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Efficiency study of testing and selection in progeny-row yield trials and multiple-environment yield trials in soybean breeding

Minghui Sun
Iowa State University

Efficiency study of testing and selection in progeny-row yield trials and multiple-environment yield trials in soybean breeding

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Minghui Sun

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Program of Study Committee:
Reid G. Palmer, Co-major Professor
Silvia R. Cianzio, Co-major Professor
A. Susana Goggi
Thomas Lubberstedt
Kenneth J. Moore
Kevin W. Matson

Iowa State University

Ames, Iowa

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ABSTRACT

Soybean [*Glycine max* (L.) Merrill] is the leading source of plant protein and oil in the world. The soybean seed has an average of 40 percent protein, one of the highest among all food crops, and approximately 20 percent oil, second only to peanut [*Arachis hypogaea* L.] among all food legumes. Soybean meal is also rich in calcium, phosphorus, and iron. Soybean is used mainly in human foods and for animals in poultry, livestock, and aquaculture feed. It has been used in many industrial products, such as pharmaceutical products and biofuel. The increased demand of soybean grain globally has fueled the research to improve the predictability of soybean yield as a means to improve the efficiency of soybean breeding.

The objective of this research is to improve the efficiencies of the soybean breeding test and selection at early stages of the progeny-row yield trials (PRYT) by experimental design and spatial modeling, and at late stages of the multiple environment trials by optimization of the multiple year and location trials (MYLT).

In the study of the efficiency improvement for test and selection in the PRYT, our results indicated that large spatial variations in soybean PRYT field could be present as evaluated by the Uniform Study conducted with two commercial lines. In this experiment, the use of the two-dimensional thin plate spline spatial model (TPS) proved to be effective in reducing the error variance and the coefficient of variability, with an improvement in relative efficiency (IRE) of 37.9%. In the Early Generation Tests, 2565 lines were evaluated within test-sets along with three checks. The TPS model also was effective in the Early Generation Tests, the IRE was 40.4%. The correlation coefficients calculated between yield

estimates obtained in the two-year Early Generation Tests and the Confirmation Study improved by 0.21 points compared to results from the non-TPS experiments. The results indicated that the use of the TPS spatial was effective in reducing the spatial variation in field tests. However, limited by the number of checks used in the research, the adjustments obtained by the TPS were not effective in increasing the selection efficiency of the Early Generation Test on the basis of the individual line performance.

In the study of the efficient improvement for test and selection in the MYLTs, our results indicated that i) the GxE was omnipresent with varied percentage of the explained variation contribution among the total observed yield variation, and the predominant source of the GxE was the GxYxL; ii) the significant presence of GxE and YxL warrants multiple-year and multiple-location trials for a sufficient predictability of the line performance in the following year. However, two-year trials should be sufficient to capture the soybean top-yielding lines. If the trials were to have a relative low presence of GxE component, one-year trial should be sufficient to identify the soybean top-yielding lines.

CHAPTER 1. LITERATURE REVIEW

Introduction of Soybean and Soybean Breeding

Soybean [*Glycine max* (L.) Merrill] is native to Asia. It belongs to the family of Fabaceae, subfamily of Papilionoideae, tribe of Phaseoleae, and subtribe of Glycininae (Palmer and Hymowitz, 2014). And it is cultivated in about 50 countries all over tropical, sub-tropical, and temperate regions (Wilcox, 2004). Brazil is the world's leading soybean producer followed by the United States, Argentina, China, India, Paraguay, and Canada (Palmer and Hymowitz, 2014). Soybean is the world's leading source of plant protein and oil. The soybean seed has an average of 40 percent protein, one of the highest among all food crops, and approximately 20 percent oil, second only to peanut [*Arachis hypogaea* L.] among all food legumes. Soybean meal also is rich in calcium, phosphorus, and iron (EI-Shemy, 2001). Soybean is used mainly in human foods, and poultry and livestock and aquaculture feed. It also is used in many industrial products, such as pharmaceutical products and biofuel.

It is widely accepted that the center of soybean origin is in northeast China with historical evidences. In this region, soybean was domesticated during the Zhou dynasty (1046–256 BC) (Ho, 1969). From about 17th - 18th century, soybean was introduced to Europe and North America (Hymowitz and Harlan, 1983; Hymowitz, 1986). Samuel Bowen, a former seaman who was employed by the East India Company, introduced soybean into North America in 1765, and in 1766, Mr. Bowen planted soybean on his garden of "Greenwich" at Thunderbolt nearby Savannah, Georgia (Hymowitz, 2004).

Glycine max (L.) Merrill is the only species cultivated within the genus *Glycine.* It is a domesticated annual species; in general, it has erect, sparsely branched, and bush-type growth habit. The leaves are pinnately trifoliolate; the colors of flower are purple, pink, or white, which are borne on short axillary racemes or reduced peduncles; the pods are straight or slightly curved mostly with one to three seeds (Hymowitz, 2004).

In the center of origin of soybean, Chinese farmers have developed landraces by saving seeds from desirable plants, and planting these seeds in the following year (Gai, 1997; Qiu et al., 1999). Because of the highly photoperiod-sensitive nature of the soybean, a cultivar of certain maturity type could only be grown successfully with about 100 km latitude range. With the extension of the growing territory from the center of origin, Chinese farmers have selected landraces based upon maturity to meet the requirements of cropping systems and food usage. Over the years, famers have selected a wide array of genes for disease resistance, seed characters, and morphological and composition traits, which have served as a global genetic diversity reservoir for modern soybean breeding (Carter et al., 2004). Collection and assembly of soybean genetic resources have been extensive by global soybean researchers. Based on the data gathered from the database maintained by Biodiversity International (www.biodiversityinternational.org) (verified July, 2013), there are more than 201,680 *Glycine max* accessions across 43 institutions in about 20 countries. There is the largest collection of soybean germplasm with about 52,776 accessions in China. US soybean researchers have explored the center and sub-centers of origin in East Asian, and have collected thousands of accessions through germplasm exchange programs. By 2001, the US Department of Agriculture (USDA) had collected more than 19,505 Asian *Glycine max* accessions including domestic and modern

breeding cultivars, genetic stocks, isolines, and registered germplasms (Palmer and Hymowitz, 2014).

It is believed that North American soybean breeding efforts began in the early 20th century. Around that time, U.S. soybean cultivars used in commercial plantings were mainly introduced from Asia, and from mass selections made by farmers and researchers from those introductions (Sleper and Shannon, 2013). With establishment of the US Regional Soybean Industrial Products Laboratory at Urbana, Illinois in 1936, soybean breeding started within the USDA-ARS and at state agricultural experiment stations (Hartwig, 1973). The Plant Variety Protection Act (PVP) passed in 1970 established the intellectual protection of crop varieties and prompted private companies to invest in soybean breeding (Pray, 1992). As a result, average on-farm soybean yields have been improved significantly due to breeding by both private companies and public research institutes. From 1924 to 2013, the national average soybean yield in the U.S. has improved from 739.8 kg ha^{-1} to 2770.7 kg ha^{-1} with an average annual gain of 23.2 kg ha^{-1} (Fig. 1), which is mostly due to genetic improvement. Diers (2013) tested 60, 59, and 49 soybean lines with release dates from 1923 to 2008 in relative maturity groups (RM) of RM II, RM III, and RM IV, respectively, (Fig. 2). He found that the rate of genetic yield improvement was 22.0 kg ha^{-1} per year on average across the three RMs.

Soybean is an autogamous crop with natural outcrossing of less than 0.5% to approximately 1% (Carlson and Lersten, 2004). As a result of self-pollination, the most commonly used breeding strategies in soybean variety development include back crossing, bulk breeding, single-seed descent, pedigree method, and early generation tests (Fehr, 1993). The choice of breeding strategies or methods depends on breeding objective,

available resources, and technologies, which in turn will impact the progress of breeding programs. A typical breeding cycle for the development of new soybean cultivars includes parental selection, crossing between desired parents to generate genetic variation in the segregating population, usually two to three generations of inbreeding, a planting for maturity classification, and then multi-stage yield trials (Fehr, 1993; Orf et al., 2004). Public release and/or commercialization begins after superior genotypes have been identified, their performance has been evaluated over a number of environments, and the seed has been increased and certified.

In modern soybean breeding, a major goal is to select cultivars with improved yield to meet the large demand for food and feed worldwide. Progress of a breeding program is determined by genetic gain per year (G_y). G_y depends on balancing several factors, and the general equation can be expressed as

$G_y = (kh\sigma_A)/y$

where k is the selection intensity, h is the square root of the narrow-sense heritability of a trait, which is the indicator of selection accuracy, σ_A is the square root of the additive genetic variation in the population, and *y* is the generation interval required to complete a cycle of breeding (Fehr, 1993).

To increase G_y, an optimal breeding program minimizes the length of breeding circle by using off-season nurseries in tropics or in temperate regions in the opposite hemisphere (Fehr, 1993). It also maximizes yield population genetic variance by using germplasm introductions and molecular technology, and maximizes yield tests and selection accuracy by using precise operational equipment, proper experimental design, and robust statistical analysis and modeling. The plant breeding literature contains numerous reports on the

procedures of improvement and effectiveness for the above factors that impact on delivering increased rates of genetic gain per year.

Yield test is one of the most important steps in a breeding cycle that provides information for prediction of future performance of the selected genotypes (Fehr, 1993). In soybean breeding, yield test and selection includes early stage yield test or progeny-row yield trial (PRYT), along with progeny-row yield selection, and later stage of multiple year and location trials (MYLT), along with the selection using the least square mean (LSM) or best linear unbiased prediction (BLUP). The objective of the PRYT is to maximize the genetic gain by selecting a subset of superior genotypes for the MYLTs in the following years. In PRYT, breeders usually make selections based on a range of agronomic traits as well as yield observed in progeny-row plots. The objective of the MYLT is to select soybean lines for commercial release in correct production regions (Cianzio, unpublished information, Iowa State University, 2008). The overall objective of this research is to improve the efficiency of the soybean breeding test and selection in the stages of the PRYT and the MYLT.

PRYT Application in Soybean Breeding

In soybean early yield test stage, there are generally large numbers of entries per cross which limit the amount of material that can be handled in replicated trials. The number of replicates and plot sizes in field design are limited due to the relatively small amount of seed harvested from each single plant. Therefore, the PRYT are commonly used in the first-year yield trial in most soybean breeding programs in North America. In PRYT, plots are single rows, planted with seed harvested from individual F_3 or F_4 plants. The observed yield values in PRYTs are the initial indicator of yield potential for F_3- or F_4-

derived lines, since each line represents a unique genetic entity from a population developed from crossing the two desired parents. A widely used PRYT test-set in North America soybean breeding programs, consists of 48 entries (45 test entries and 3 checks) with field lay-out of 12 columns in width and 4 ranges in depth (Fig. 3). C1, C2, and C3 stand for checks. To accommodate mechanic operation, checks usually are placed in the beginning of the test-set. The test plots are unreplicated one-row plots, usually 1.5 m in length with a 0.8 m space between rows planted at one location. Each year, within a typical breeding program, there are hundreds of crosses made giving rise to F3-derived populations, which are tested in the PRYT. Only a small fraction of the lines in each population with yield over the average of commercial checks about 10-15% is selected from the PRYT. Some populations may be discarded completely (Cianzio, unpublished information, Iowa State University, 2008). Therefore, a tremendous amount of resources are invested in the maintenance and evaluation of PRYT plots; so that, it is vital that proper field experimental design and suitable statistical analysis for the PRYT are used.

The success of the PRYT depends upon the accurate evaluation of heterogeneous populations and the ability to distinguish the genetic differences among genotypes in an unreplicated one-location test, and the persistence of those differences in later generations. The PRYT in self-pollinating species, as a means to identify superior genotypes, has been reported in different crops with varying success. Dahiya et al. (1984) evaluated four F_3 populations of chickpea (*Cicer arietinum L.*) in single progeny-row plots. Within each population, they selected 10 high yielding, 10 low yielding, and 10 randomly selected lines, along with 10 lines by visual yield selection. The four groups of lines were bulk-harvested, and further evaluated for yield in replicated trials at three locations. They found that the

yields of the bulks of the selected 10 high yielding lines and 10 low yielding lines correlated well with that from replicated trials at three locations, whereas, the yield of the bulk from visual selection was not superior to the bulk of the randomly selected lines. They concluded that an early generation yield-testing selection procedure based on single progeny-row plot test was effective, whereas, visual selection was in-effective for yield selection. DePauw and Shebeski (1973) assessed the yield correlation and regression coefficients between wheat (*Triticum aestivum L.*) yield estimated on F_3 progeny-row yield test and the related bulk means of F_4 and F_5 from multiple location tests. They concluded that progeny-row yield trials could discriminate among F_3 lines for heritable quantitative differences. However, in the research with cowpea (*Vigna unguiculata* (L.)), Padi and Ehlers (2008) did not find a correlation between yield based on unreplicated F_3 individual plant data tested at one-location and their yield performance in F_4 bulks tested at three locations. They concluded that early generation selection for yield was ineffective in cowpea based on single-plant plot yield tests.

In soybean, Horner et al. (1957) and Brim et al. (1961) found that a large proportion of the genetic variance for yield was additive. This suggests that an individual soybean line with superior combination of yield genes should have high yield potential, and the high yielding heterogeneous populations with F3-derivatives would be indicative of the high yielding pure lines that could be derived from that cross. Therefore, single-row PRYT could be a reliable predictor of yield in multiple environment advanced yield trials in late generations.

However, reports from early generation studies, while generally favorable, gave mixed results. Cooper (1990) reported that based on the reduced number of F_2-derived

lines per cross, and the use of a single location, single replication data for the selection in $F_{2,3}$ through $F_{2,3,4}$, eight high-yielding soybean cultivars were released. Streit et al. (2001) reported that soybean seed yield selection based on PRYT was as effective as based on replicated multiple environment tests for all four populations used in their research. Hegstad et al. (1999) concluded that PRYT test could make the progress in identifying elite soybean lines with high genetic potential. However, the correlations of yield in PRYT and replicated tests in only two out of five of their populations were significantly positive.

The varying levels of effectiveness reported in different studies might be the result of differences in selection procedures and criteria, population heterozygosity, heritability of traits, and statistical analyses. Based on theoretical studies of the effectiveness of early generation selection under PRYT in self-pollinated crops, Bernardo (2003) concluded that early generation selection was expected to be effective, unless in practice, non-genetic effects were large relative to true genetic merits of the test lines. Yang (2009) suggested that early generation selection should be used for populations or traits with little non-additive effect, coupling linkage, and high heritability. Therefore, the understanding of the factors, that contribute to yield variation, and effectively controlling non-genetic effects are the keys for the success of the PRYT.

Non-genetic Factors Contributing to Yield Variation and Selection Accuracy in PRYT

The grain yield potential of the crop is determined by genetic, non-genetic or environmental factors, and the interactions between them (Fehr, 1993). The environmental factors can be partitioned into: soil types, climatic factors, pest conditions, and operational management. Grain yield responds to non-genetic factors such as spatial and temporal heterogeneity of environmental factors, changes in management, and

interactions among these factors (Bresler et al., 1981; Moore and Tyndale-Biscoe, 1999; Kaspar et al., 2003; Bakker et al., 2005). Based on the analysis of regional wheat yield data at NUTS2 and NUTS3 level for most of the EU-countries, Bakker et al. (2005) concluded that i) the yield trends in wheat were closely negatively correlated to radiation and mean annual temperature, though the positive effect of radiation and temperature on plant photosynthesis, because the high radiation and mean annual temperature might cause water supply stress; ii) the yield variations and trends in wheat were poorly related to rainfall, this was explained as the increasing risks of pests and diseases from rainfall counterbalancing the positive effect of rainfall on crop growth.

It is commonly accepted that soybean yield in a test has high variability across fields (Paz et al., 1998). Based on analysis of data over three years within a 16-ha Iowa field, Paz et al. (1998) found that variations in soil water holding characteristics, which caused the spatial patterns in terms of drought stress, could explain 69% of the variability in soybean yield. From the simulation study using CROPGRO-Soybean model (Jones et al., 2003), Paz and Batchelor (2000) showed similar results.

In micro-environments, such as an experiment field, the variation in observed yield of soybean comes also from the genetic variation of test lines and the variation of non-genetic factors, typically field spatial patterns and experimental error (Bernardo, 2003; Zimmerman and Harville, 1991). The variation due to non-genetic effects could be confounded with the genetic variation of yield and its components (Becker, 1995). A major non-genetic effect is spatial trend within the test field, which could be caused by soil heterogeneity, agricultural practices, pest pressure variation, and other non-genetic factors (Gilmour et al., 1997). Field spatial trend usually is measured as a substantial yield

correlation among neighboring plots (Brownie et al., 1993; Federer, 1956). Significant spatial trends could have large effect on the ranking of test lines on the basis of their genetic potential for yield, and hence, could bias the accuracy of selection (Brownie et al., 1993; Stroup et al., 1994).

Non-genetic variances in unreplicated PRYT present great challenges on the estimated genetic values of the test lines. Breeders try to minimize those effects on experimental units in unreplicated trials through standard operational procedures, robust field experimental designs, such as blocking and random or/and systematic placement of common checks, and various spatial analyses. The purpose of blocking is to increase precision by evaluating treatments in similar environmental conditions within a block. However, even with highly controlled operational procedures with precise mechanic systems, there might still be considerable variation among plots within a block in soil properties e.g. fertility, drainage, pest pressure, etc. The greater the heterogeneity within blocks, the greater the variation of estimates of line true genetic effects, and the poorer the precision of the selection. Spatial variations are likely to complicate estimation of yield and its components (Becher, 1995; Kirk et al., 1980). Significant spatial heterogeneity could impair the standard statistical inference techniques, which assumes that the observations are independently and identically distributed with a random error variance. As a result, there might be dramatic reductions of the accuracies in estimations of genetic variation and genetic values of the test lines. Therefore, proper experimental designs and spatial analyses could be a better option (Brownie et al., 1993; Magnussen, 1993; Stroup et al., 1994; Rosielle, 1980).

Increasing efficiency by using spatial analysis in unreplicated yield trials has been reported. In sugarcane, Stringer and Cullis (2002) found that spatial design and analysis could account for spatial trends and plot competition in non-replicated sugarcane early-stage trials and could improve efficency of analyses by better partitioning of the variance components for blocks and residual effects. Edmé et al. (2007) found that the spatial analyses improved selection accuracy based on non-replicated early-stage sugarcane field experiments and maximized genetic gain. However, choosing the appropriate spatial model for the data sets still remains a challenge.

Experimental Designs and Spatial Statistical Modeling to Account for Spatial Variation in PRYT

As discussed above, the variability caused by field spatial heterogeneity needs to be properly adjusted by spatial modeling in the analysis of the PYRTs. Here I discuss a few of the most commonly used methods in accounting for spatial variation in unreplicated agricultural trials, which include ordinary linear variance models with row-column model (RC) and augmented randomized complete block design (ARCBD), nearest neighbor adjustment, autoregressive models, and the newly introduced two-dimensional thin plate spline regression model (TPS) to estimate spatial effects.

Ordinary Linear Variance Models. Traditional methods in adjusting field spatial variation by experimental design include random complete block design (RCBD). The total field available for an experiment is divided into blocks. Assumptions are made that within each block the environmental effects are homogeneous and can be ignored. The experimental units are randomly assigned to different blocks with replicates. Environmental effects are adjusted by including block effects in the model. The RCBD can

be effective, but has limited application since the design of blocks can be uncontrollable and the assumption of uniform environmental effects within blocks is almost always violated. The RCBD was later expanded into two-dimensional block design, this is the well-known row-column (RC) design. Yates (1939) and Cochran and Cox (1957) first introduced lattice square designs to eliminate spatial variation from row and column aspects in test line comparisons. Kempton (1985) and Robinson et al. (1988) found that the RC model increased relative efficiency significantly when compared to RCBD model. The RC model assumes that the spatial effects follow vertical and/or horizontal trends, and there is no interaction among the two directions, which is hardly the case. The trend in field spatial effects is almost unpredictable. And, when there is interaction between lattices, the mean square error from the RC model can be inflated and leads to false tests of treatment effects.

The RCBD requires replicates across blocks for spatial adjustment of block effects and accurate estimation of treatment effects. However, in the PRYT, replicates are often not possible. Federer (1956) proposed augmented random complete block design (ARCBD) for spatial adjustment across blocks in non-replicated trials. Although different types of ARCBD exist, in general, they use two types of treatments as replicated reference units (i.e. checks) and non-replicated experimental units. The experimental units are partitioned equally into groups across the entire experimental field. The reference units are replicated randomly in each of the treatment groups to capture the field spatial trend across the whole field. The performance of experimental units are adjusted by that of the reference units based on the assumption that reference units react to the factors causing spatial trend in a similar manner in all the experimental units. The ARCBD was effective in

accounting for field spatial trend and improving accuracy of estimating treatment effects and selection of genotypes (Rosielle, 1980; Magnussen, 1993; Stringer and Cullis, 2002).

Nearest Neighbor Adjustment. The unexpected field heterogeneity requires more sophisticated spatial modeling that could effectively capture local and universal trends instead of simple assumptions as in RCBD or RC. Papadakis (1937) proposed an ad hoc method that adjusts the performance in a plot by the performance in its neighboring plots. For the yield record in each plot, a covariate is generated by averaging the yield residuals in all its neighboring plots, and the covariate is included in the model as the adjustment of the local spatial effects (Papadakis, 1937). Bartlett (1938) evaluated Papadakis' method in more detail. According to Bartlett (1938), Papadakis' method is only an approximation and lacks mathematical validity, and the method requires a sufficiently large number of plots within each block to get useful results. Pearce and Moore (1976) reported that the accuracy of estimates of treatment effects increased significantly when applying Papadakis' method. Due to his concern about obtaining adjustment covariates from the observations, Bartlett (1978) later suggested an iterative method that updates Papadakis' covariates. The treatment effects are re-estimated in each step of iteration and the adjustment procedure continues until the estimates stay constant (converging). The iterative method suggested by Bartlett (1978) was more effective than Papadakis' method without iteration. In either method, the adjustment was calculated only through the observed data, and the field spatial covariates were not incorporated into the analysis. Based on Papadakis (1937) and Bartlett (1938, 1978), Taye and Njuho (2007) recently proposed an improvement on Papadakis' covariate by introducing a kriged covariate based on field spatial information and by assigning equal weights to neighboring plots regardless of the distance from the

current plot. By evaluating both simulated and real data-sets, Taye and Njuho (2007) demonstrated that the improved Papadakis covariate was more effective in accounting for spatial variation than the conventional Papadakis covariate.

Autoregressive Model. Although adjustment of spatial effects using neighboring plot information could effectively account for local field spatial trend, it is usually the case that global trend is phenomenal and is greatly confounded with treatment effects. Modeling local spatial variation using neighboring information alone could not effectively account for variation due to the global field spatial trend. The "a posteriori" modeling has been proposed to capture both global and local trends (Besag and Kempton, 1986; Gleeson and Cullis, 1987; Zimmerman and Harville, 1991; Cullis and Gleeson, 1991). In such models, the global trend is modeled by the expectation, and local trend is modeled through random effects with spatial correlation structure, in which the autoregressive structure is most widely used. In contrast to the Papadakis' modeling, autoregressive models assume that the performance of the current plot correlates with other plots depending on the spatial distance between them. Results from simulation and field datasets have shown that models with autoregressive structure generally perform better in accounting for spatial variation than random block design, and models with two-dimensional correlation structure are generally the most effective (Grondona et al., 1996).

Two-dimensional Thin Plate Spline Regression Model (TPS). Although "a posteriori" spatial modeling was shown to be more effective than block design in accounting for local and global field spatial trends, these methods have several drawbacks, particularly in PRYT field lay-out. First, generally many parametric correlation structures are available, but there is no general rule to select the best model for a specific dataset.

Second, evaluation of a model adequacy and comparison of different models are not straightforward and often highly dependent on model assumptions (Grondona et al., 1996). Third, in general, the PRYT field lay-out in soybean breeding yield trial is that the lines derived from the same crossing population are tested within the same test sets. The genetic relatedness of the lines could manifest correlation among neighboring plots calculated by semi-variogram which is the measurement of spatial trend. Semi-parametric or non-parametric models usually require little or no parametric assumptions and the inferences are mostly learned from the data. Recently, the TPS became popular in spatial analysis of yield-trial data. Splines are piecewise functions that are fit through control points referred to as knots. This allows complex functions to be partitioned into relatively simple functions joined by knots. Each spatial effect can be estimated as a function of its surrounding knots by using a localized interpolation function (Bazen and Gerez, 2002; Bookstein, 1989; Robbins et al., 2005). Compared with block designs and autoregressive models, the TPS modeling of spatial effects is free of parametric assumptions and hence is robust and applicable to general datasets. The TPS spatial model has been effective in engineering for modeling deformation in two-dimensional planes (Bazen and Gerez, 2002). Robbins et al. (2012) introduced the TPS to North American maize non-replicated yield trails to model two-dimensional spatial patterns of experiment fields, and they found that the two-dimensional TPS model was effective and robust at reducing the error variance by correction of spatial trend for an unreplicated trial based on both simulation data and actual maize trial data. This could provide an opportunity to improve genetic selection by accurate partitioning of spatial and genetic effects.

Genotype by Environment Interaction and Predictability of Soybean Line Performance Based on Multiple Year and Location Trials

In breeding and improvement of crops, genetic gain per-year determined for a target trait is the primary measure of the success of the program. Among many sources of variation that could determine the success of the breeding program, genotype x environment interaction (GxE) present in multiple environment trials is one of the major factors that impact genetic gain (Fehr, 1993). From a statistical point of view, GxE is defined as change in rank of the line performances across locations, namely crossover GxE, or change in the magnitude of difference of line performances across test locations, namely non-crossover GxE (Haldance, 1947; Mather and Caligari, 1976; Gregorius and Namkoong, 1986; Baker, 1988, 1996). To achieve high genetic gain for low heritability traits, such as yield, plant breeders designed testing and selection strategies that might help to account for GxE effect (Fehr, 1993). One approach is the use of multiple year and location trials (MYLT) that will allow measuring GxE effects and more precisely evaluate line performance.

From theoretical studies in the MYLT, effect of GxE could be partitioned into genotype x location interaction (GxL), genotype x year interaction (GxY), and three-way interaction of genotype x year x location (GxYxL) (Comstock and Moll, 1963; Annicchiarico and Perenzin, 1994; Yan and Rajcan, 2003). To estimate GxL effects, trials are conducted in multiple locations during a single year. Similarly, to estimate GxY effects, multiple-year trials are conducted, and by having multiple years and locations, effects of GxYxL are estimated (Yan and Rajcan, 2003). It is assumed that the MYLT could provide more accurate estimates of true genetic values of the test lines, and more precisely predict the

line performance in years and locations in which the lines will be planted in the future (Yan and Rajcan, 2003). The published information comparing the efficiency of one-year multiple-location trials *vs* MYLTs is limited, and results were contradictory depending on the crop species and within crops also depending on maturity group of the test lines (Cross and Helm, 1986; Gellner, 1989; Bowman, 1998; Yan and Rajcan, 2003). Based on the analysis of combined 10-year data of the Ontario Soybean Variety Trials in four locations from 1991 to 2000, Yan and Rajcan (2003) could not partition GxE effect into GxY, GxL, and GxYxL limited by the attributes of the data. However, based on the Pearson product-moment correlation coefficient (r) between line performance in one year estimated using one-year multiple-location trials and that estimated in the next year multiple-location trials, they concluded that one-year multiple-location trial was sufficient to predict soybean line performance in the next year. Using a balanced sub-set of data with barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), oat (*Avena sativa* L.), soybean (*Glycine max* (L.) Merr.), and wheat (*Triticum aestivum* L.) from the North Carolina Official Variety Trials, Bowman (1998) found that GxL was not present in all six crops, GxY occurred with barley and cotton, and GxYxL was important with barley, wheat, and soybean at RM group VI. Based on the probability of the turnover for the top-yielding lines, he concluded that all six crops needed two-year multiple-location trials to ensure the sufficient prediction efficiency for top-yielding lines in the next year, except mid-season corn hybrids which only needed one-year multiple-location trials. Based on the analysis of spring wheat and oat data from the South Dakota Cultivar Test from 1972 to 1987, Geller (1989) concluded that one-year multiple-location trials were equally good for prediction of the next year performance as that based on previous two- or three-year trials.

Dissertation Organization

In the soybean researches reported here, and as an effort to improve the efficiencies of the tests and selections in PRYTs and MYLTs, five studies were conducted. They were i) the Uniformity Study conducted in 2012; ii) the Early Generation Tests conducted in 2010 and 2012; iii) the Confirmation Study conducted in 2011; iv) the Predictability Study conducted in years 2009, 2010, 2011, and 2013, and v) the Spatial Study conducted in 2013. The objectives of the first three studies were to i), quantify the non-genetic component of yield variation in PRYT; ii), compare relative efficiency of the TPS spatial model over standard analysis of variance; iii), and assess the application of the TPS spatial model to improve selection accuracy at early generation trials, namely PRYTs. The results are presented in Chapter 2 of the dissertation. The objectives of the Predictability Study were to i) quantify the GxL effect, the GxY effect, and the effect of the three-way interaction of the GxYxL using a wide arrange of years and locations to evaluate elite soybean lines; and ii) evaluate the prediction efficiency of single-year multiple-location yield trials. The results are presented in Chapter 3 of the dissertation. The objectives of the Spatial Study in 2013 were to i) determine, whether by increasing the number of checks and with random arrangement within test-sets in the PRYT, the TPS could improve the selection efficiency on the basis of individual line performance; and ii) determine, whether the application of more checks and with random arrangement within test-sets, the TPS could further increase the estimation accuracy in the PRYT on the basis of population mean performance.

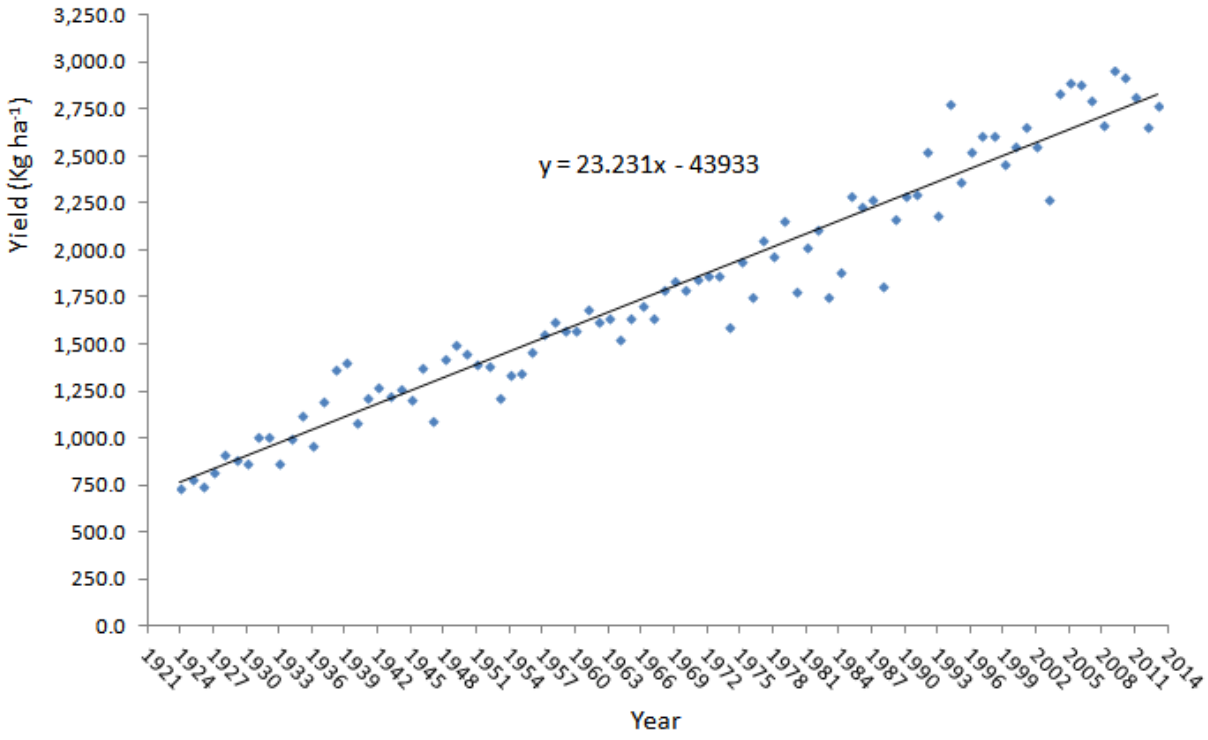

Fig. 1 Soybean historic yield data (kg ha^{-1}) from 1921 to 2014 in the United States (USDA data base: *http://www.usda.gov/wps/portal/usda/usdahome?navid=DATA_STATISTICS*).

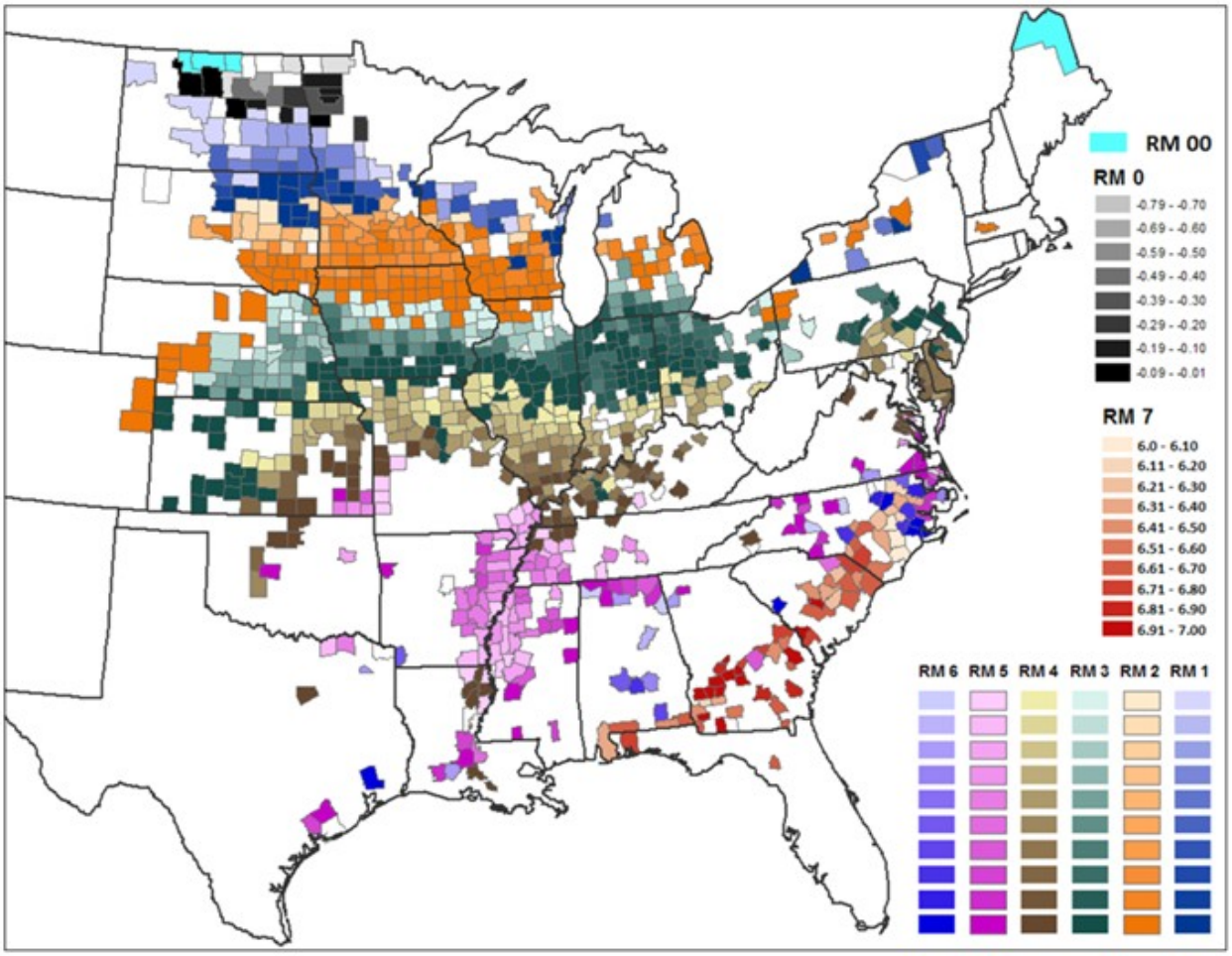

Fig. 2 Soybean relative maturity groups in the United States (Kevin Matson, personal communication, Monsanto Inc. 2012).

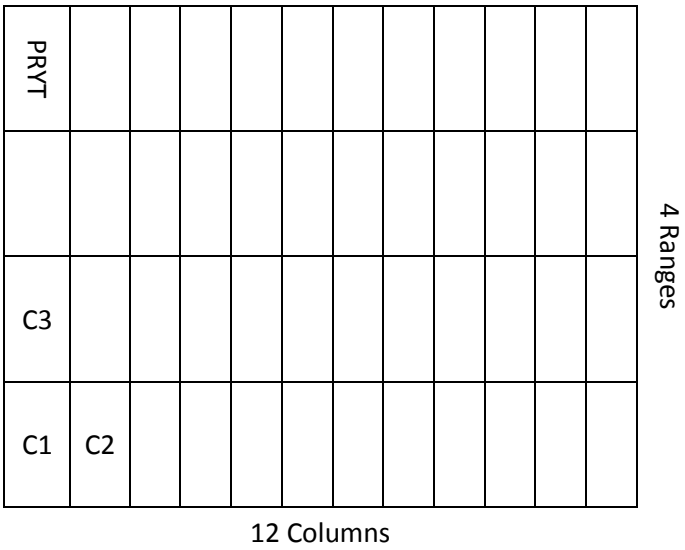

Fig. 3 Typical field lay-out of soybean PRYT test-set in North America. Within a test-set, 48 entries (45 test entries and 3 checks) were tested under field lay-out of 12 columns in width and 4 ranges in depth. C1, C2, and C3 stand for checks (Cianzio, unpublished information, Iowa State University, 2008).

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CHAPTER 2. THIN PLATE SPLINE SPATIAL MODEL USED AT EARLY STAGES OF SOYBEAN BREEDING TO CONTROL FIELD SPATIAL VARIATION: THE STUDY OF TEST EFFICIENCY IMPROVEMENT USING THREE CHECKS WITHIN TEST-SETS

M. Sun[1*], S. A. Goggi[2], K. Matson[1], R. G. Palmer[2], K. Moore[2], and S. R. Cianzio[2*]

[1] Monsanto Inc., St. Louis, Missouri 63017, USA

[2] Department of Agronomy, Iowa State University, Ames, Iowa 50011-1010, USA

Address correspondence to M. Sun at 3302 SE Convenience Blvd., Ankeny, IA 50021, USA. E-mail: msun0203@yahoo.com

Address correspondence to Dr. Cianzio at 2107 Agron. Hall, Ames, IA 50011-1010, USA. E-mail: scianzio@iastate.edu

ABSTRACT

The early selection of superior plants with high-yielding potential is a major goal in soybean breeding programs. The progeny-row yield trial (PRYT) is usually conducted as a means to assess the yield potential of F_3-derived progenies. The yield variation observed in the PRYT is the final result of the line genotypic merit, the field spatial pattern, and the experimental error. The spatial variation in field tests could confound the estimates of genetic merits, and might induce errors in selection of superior lines, thus decreasing the estimates of genetic gain per year. The objectives of this research were to: i) quantify non-genetic yield variation in a soybean breeding PRYT; and ii) determine the efficiency of the Thin Plate Spline (TPS) spatial model in adjusting yield due to variation caused by field spatial pattern. The third objective was to evaluate if the use of the TPS model could improve the selection accuracy of PRYT un-replicated yield tests. Three experiments were conducted, a

Uniformity Study, an Early Generation Test, and a Confirmation Study. The TPS model first was described by Bookstein in 1989. Our results indicated that large spatial variations in soybean PRYT field could be present as evaluated by the Uniformity Study conducted with two commercial lines. In this experiment, the use of the TPS proved to be effective in reducing the error variance and the coefficient of variability, with an improvement in relative efficiency (IRE) of 37.9%. In the Early Generation Tests, 2565 lines were evaluated within test-sets along with three checks. The TPS model also was effective in the Early Generation Tests, the IRE was 40.4%. The correlation coefficients calculated between yield estimates obtained in the two-year Early Generation Tests and the Confirmation Study improved by 0.21 points compared to results from the non-TPS experiments. The results indicated that the use of the TPS spatial model was effective in accounting for some of the spatial variation in field tests. However, limited by the number of checks used in the research, the adjustments obtained by the TPS were not effective in increasing the selection efficiency of the Early Generation Test on the basis of the individual line performance. Research is in progress to determine if by increasing the number of checks within the test-set in the early generation tests, the TPS spatial model could improve the selection efficiency of the lines evaluated in the early generation tests.

Keywords Progeny-row yield trial, genetic gain, two-dimensional thin plate spline, best linear unbiased prediction, soybean breeding

Abbreviations and Nomenclature (PRYT) progeny-row yield trial; (CV) coefficient of variation; (SRMSD) square root of mean square deviation; (SRMSE) square root of mean

sum of squares for error; (TPS) two-dimensional thin plate spline; (IRE) improvement in relative efficiency; (IQR) interval quartile range; (yld_adj) yield with the TPS spatial effect adjustment; (BLUP) Best linear unbiased prediction; (RM) relative maturity.

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INTRODUCTION

The major goal in soybean breeding programs is to select cultivars with superior yield. Development of new cultivars in a breeding cycle involve parental selection, crossing between the desired parents to generate genetic variation in the segregating population, two to three generations of inbreeding, a planting for maturity classification, and multi-stage yield tests conducted over locations and years (Fehr, 1993; Orf et al., 2004). Public release and/or commercialization begin after the superior genotypes have been identified, and their performance has been evaluated over a range of environments. One of the most important steps in the breeding cycle is the yield test conducted at early stages of the program.

Soybean yield tests at early stages generally consist of progeny-row yield tests (PRYT) (Fehr, 1993). Plots in the tests are usually single-rows planted with seed from an individual plant. The limited seed numbers produced by an individual plant imposes limitations to plot sizes and also to number of replicates. The progeny-row yield tests are the initial indicator of yield potential for F_3- or F_4-derived lines, since each line represents a unique genetic entity from a population developed by crossing the two desired parents. A widely used layout of PRYT fields in North American soybean breeding programs consists

of un-replicated one-row plots, usually 1.5 m in length with a 0.8 m space between rows planted at one location (Cianzio, unpublished information, Iowa State University, 2008).

The use of PRYT to predict yield potential at early generations has shown to be effective in predicting the yield potential for later plantings conducted at multi-location tests (Hegstad et al., 1999; St. Martin et al., 1990). Bernardo (2003) conducted a simulated study for self-pollinated crops and concluded that early generation tests and PRYT-based selection of lines were expected to be effective in predicting performance of genotypes, unless non-genetic effects were large relative to the true genetic merits of the tested lines. Bernardo's observations suggest that test size may be an important factor that could affect the prediction value of the early generation tests. According to Bernardo (2003), the observed yield variation in PRYT results from the genotypes of the soybean lines, and the addition of variable non-genetic effects, such as field spatial patterns, and experimental error. The variation due to non-genetic effects could be a confounding factor in determining the genetic variation in yield of the test lines (Becker, 1995).

The heterogeneity and spatial trend within the test field, caused by factors such as soil heterogeneity, agricultural practices, pest pressure variation, among others, may be contributors to the non-genetic effects (Gilmour et al., 1997). The field spatial trend is usually measured as a yield correlation among plots (Brownie et al., 1993; Federer, 1956), and this can have large effects on line rank and hence bias the accuracy of selection (Brownie et al., 1993; Stroup et al., 1994). Lussenden (personal communication, Monsanto Inc., 2011) observed that yield variation caused by non-genetic effects could be as high as 383.3 kg ha^{-1} in soybean PRYT fields in Southern MN, with a mean performance of lines of 1748.5 kg ha^{-1}.

The experimental error is a random variable contributing to bias the estimates of genetic superior lines. The spatial pattern in yield is, however, often predictable, and may be removed from the observed yield by proper experimental design used in conjunction with spatial models (Federer, 1956; Rosielle, 1980; Brownie et al., 1993; Magnussen, 1993; Lin et al., 1993; Stroup et al., 1994; Becher, 1995; Stringer and Cullis, 2002; Robbins et al., 2012; Edmé et al., 2007).

For non-replicated experiments with a large number of lines under evaluation as is often the case in PRYT, the augmented design (Federer, 1956) along with proper modeling (Scott and Milliken, 1993) has been effective in removing the spatial trend in one dimension (Federer, 1961). Spatial trends in an experimental field, however, are not limited to a single dimension, and the augmented design may not be sufficient to capture spatial trends in two dimensions (Federer, 1961). The Thin Plate Spline (TPS) spatial model can be used to address this problem where splines are piecewise functions fitted through control points, referred to as knots, and thereby allow complex functions to be approximated by relatively simple functions connected by knots (Bookstein, 1989; Bazen and Gerez, 2002; Robbins et al., 2012). The TPS spatial model has been effective in engineering for modeling deformation in two-dimensional planes (Bazen and Gerez, 2002). Robbins et al. (2012) introduced the TPS to maize non-replicated yield trails and validated the use of the TPS using simulated data. The authors indicated that the TPS effectively dissected the spatial trend from the genetic effects, and reduced the error variance by modeling the spatial variation.

There are no published studies to investigate the effect of the TPS spatial model in capturing and removing field spatial trend in PRYT for soybean yield. The objectives of this

research were to quantify non-genetic yield variation in a soybean breeding PRYT, and determine the efficiency of the TPS spatial model in adjusting yield due to variations caused by field spatial pattern using a standard analysis of variance. The third objective was to evaluate if the application of the TPS model could increase the selection accuracy in PRYT un-replicated yield tests.

MATERIALS AND METHODS

The research consisted of three separate experiments: 1) the Uniformity Study; 2) the Early Generation Tests; and 3) the Confirmation Study. The Uniformity Study was performed to quantify the possible yield variation within a PRYT test-set, and to determine the efficiency of the TPS spatial model by the improvement in relative efficiency (IRE) between the actual yield test and the adjusted yield test. The Early Generation Tests were designed to assess the efficiency of the TPS spatial model by obtaining a correction factor for the spatial effects. The Confirmation Study was designed to assess the efficiency of the TPS spatial model by comparing the performance of the bi-parental populations and the individual lines evaluated in the Early Generation Tests and their later evaluation in multiple location tests. The line and population performance estimates obtained from the Confirmation Study were assumed to provide closer estimates to the true genetic performance of the lines, since the estimates were derived from replicated multi-location field tests.

In the conduct of the three experiments, field plots were planted and harvested mechanically, utilizing computer technology to automatically record grain harvest and grain moisture. The agronomic data recorded in each individual plot were relative

maturity (RM) at reproductive stage R7 (Fehr et al., 1971), and grain yield measured in kg ha^{-1} at reproductive stage R8 (Fehr et al., 1971) with a moisture correction factor to express yield at 13.5% moisture content. The individual plots of each experiment were each harvested in bulk.

Uniformity Study

Two lines, MON12 and MON14, were selected for this study. The lines were commercial pure lines with stable yield performance from 2010 to 2012 (Lussenden, personal communication, Monsanto Inc. 2012). MON14 yields on average 190.1 kg ha^{-1} more than MON12 based on 737 within test-set comparisons at 199 locations evaluated from 2010 to 2012 in Monsanto Inc. yield tests.

In 2012, the Uniformity Study was conducted at the Monsanto Inc. soybean research station located in Southern MN. The soil at the research location is a silt clay loam, and the sowing date was June 11. Each line was planted in 48 row-plots, arranged in a grid four ranges deep and 12 columns wide. The 48 row-plots of each line were considered as a separate test-set. Each test-set was planted in a block, according to the field layout of a randomized complete block design (RCBD) with four blocks and two replicates within each block. The row-plots within test-sets were 1.5 m in length, spaced 0.8 m between rows, planted with 40 seeds resulting in a sowing population of 27 seeds m^{-1}.

Early Generation Test

The Early Generation Tests were conducted in 2010 and in 2012 at the Monsanto Inc. soybean research station located in Central IA. The soil at the research location is a

sandy clay loam. The sowing date was May 5 in 2010, and May 10 in 2012. The weather observed between the two years was very different, particularly in reference to rain fall received during the growing seasons (Table 1). Nineteen bi-parental populations each represented by 135 F_3-derived lines in $F_{3:5}$ were evaluated every year for a total of 2565 $F_{3:5}$ lines. The 135 lines per population were randomly divided into three groups of 45 lines each, to which three checks were added, bringing the total to 48 entries per test-set. Each of the 19 bi-parental populations was represented by three test-sets as three replicates. Each of the three checks formed a check-set with three replicates. Each check-set contained one check replicated 46 times, and two other checks replicated only once. Total number of checks used in the experiments was three.

According to the field layout of the RCBD, the 19 test-sets and the three check-sets were assigned to three blocks. Within a block, the test-sets and the check-sets were arranged randomly. Similar to the Uniformity Study, the 48 entries within a test-set also were arranged in a grid four ranges deep and 12 columns wide. To accommodate the auto selection controlled by the computer based on yield advantage to average yield of the checks during mechanical harvest, the three checks were placed always at ranges 1 and 2 of column 1, and the third check at range 1 of column 2. The same check arrangement was used also within check-sets. Row-plots were planted at 40 seeds per plot in 1.5 m row length, spaced 0.8 m between rows resulting in 27 seeds m^{-1}.

Confirmation Study

The seeds of each of the 135 $F_{3:5}$ lines per population (19 populations) harvested in bulk from the 2010 Early Generation Test, were used to plant the Confirmation Study in

2011. The study was conducted at four locations (Central IA, Northwest IA, Eastern IA, and Southwest IA) at the Monsanto Inc. research sites. The soil at four locations is a sandy clay loam, a clay loam, a silt clay loam, and a clay loam, respectively. The sowing date was May 10, May 9, May 7, and May 10, respectively. The 2565 lines were classified into three maturity groups, defined on the basis of the RM recorded in the 2010 PRYT. The group with RM from 2.0 to 2.4 was composed of 405 lines and identified as F24S. The second RM group was from RM 2.5 to 2.8, composed of 1215 lines, and identified as F26S. The third group was identified as F29S, had RM from 2.9 to 3.1, and was composed of 945 lines. Within each RM group, 45 F_3-derived lines in $F_{3:6}$ were assigned randomly to one test-set, and five checks were added, bringing the total to 50 entries per test-set. Within a test-set, the 50 entries were randomly planted in the field in a grid five ranges deep and 10 columns wide. Plots were two-row plots, 3.7 m in length, and 0.8 m between rows resulting in a sowing population of 30 seeds m^{-1}.

Statistical Analysis

The assumptions of normality and homogeneity of variance in the error term of yield data from each of the three experiments were evaluated before any parametric statistical analysis was conducted. In all experiments, the yield data were continuous with near normal distribution based on the test of QQPLOT in the PROC UNIVARIATE procedure of SAS version 9.3.2 (SAS Institute, Cary, NC).

Box-plots based on the interval quartile range (IQR) method (Tukey, 1977) were implemented for outlier identification. IQR was calculated on a whole experiment base for the Uniformity Study and the Early Generation Test. IQR for the Confirmation Study was

calculated for each individual location. On the basis of the research conducted by Tukey (1977) and Eo et al., (2012), an observed yield value is treated as an outlier when

$$y < q1 - 1.5*iqr \text{ or } y > q3 + 1.5*iqr \quad [1]$$

where y is the observed yield; q1 was the 25% sample quartile, q3 was the 75% sample quartile, and IQR was the difference between q3 and q1, for a given data set of observed yield values. An outlier is, therefore, treated as a missing value, otherwise, to avoid distorting the statistical inference. The yield data from each of the three experiments was analyzed using the SAS 9.2.3 statistical package (SAS Institute, Cary, NC), and R FIELDS package (Nychka, 2013).

In the Uniformity Study, the yield variation observed within a test-set was assumed to consist of the field spatial patterns and the experimental error. The variation observed across the whole experimental field was assumed to consist of the field spatial pattern, the experimental error, and the genetic variation between the two test lines. The yield data were first fitted into the general linear model with line as a fixed effect

$$y_{ijk} = \mu + v_k + e_{ij} \quad [2]$$

where y_{ijk} is the observed yield of the line k at range i and column j; μ is the overall mean; v_k is the line k effect; and e_{ij} is a random error having a normal distribution $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. Assuming the estimated residual of each plot, from fitted model, as a combination of spatial patterns and experimental error, the TPS was implemented with the residuals to separate the spatial patterns from the experimental error.

The TPS model is a semi-parametric spatial model (Bookstein, 1989). Under the TPS model, the spatial trend effect at each progeny-row plot can be estimated as a function

of its neighboring check plots, referred to as knots, by using a localized interpolation function (Robbins et al., 2005). The resulting TPS model corresponded to a mixed linear model for yield of line i in range j and column k given as

$$y_{kijl} = \mu + \sum_{l=1}^{n} W_{ijl}\beta_l + v_k + r_i + c_j + e_{kijl} \quad [3]$$

where μ is the overall mean; β_l is a fixed effect for the l-th knot; W_{ijl} is the weight for the l-th knot at range i and column j; v_k is the genetic effect for the k-th line; r_i is the random effect for the i-th range; c_j is the random effect for the j-th column; and e_{kijl} is a random error for the plot at range i and column j with $e_{kijl} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2 (Robbins et al., 2012).

The weight W_{ijl} is defined as

$$W_{ijl} = \frac{1}{\left\|R_l - R_i, C_l - C_j\right\|}, \quad [4]$$

where R_l and C_l are the range and column for the l-th knot; respectively; and R_i and C_j are the range and column for the plot on which the spatial effect β_l will be estimated, respectively, the expression $\|a, b\| = \sqrt{a^2 + b^2}$ is Euclidian distance. The spatial effects were estimated using the R package FIELDS of version 6.9.1 (Nychka, 2013).

The spatial patterns obtained from the Uniformity Study were used to adjust the observed yield on the basis of progeny-row in the Uniformity Study. The observed yield after adjustment of the spatial effects predicted from the TPS was denoted as yld_adj. The same notations were applied in the Early Generation Tests as well.

In the Uniformity Study, the analysis of variance for a RCBD was conducted with the following model

$$y_{ij} = \mu + v_i + b_j + vb_{ij} + e_{ij} \quad [5]$$

where y_{ij} is either the yield or the yld_adj of the line i in the block j; μ is the overall mean; v_i is the line i effect; b_j is the block j effect; vb_{ij} is the line i and block j interaction effect; and e_{ij} is a random error having a normal distribution $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. Data were analyzed using the PROC GLM procedure of SAS version 9.3.2 (SAS Institute, Cary, NC). The mean difference between the lines was tested for significance by Fisher's least significant difference test (LSD) (Fisher, 1935).

In the Early Generation Tests, the yield data set, containing only the checks, was first fitted into the same general linear model with checks as fixed effects as described in equation[2], where y_{ijk} is the yield of the check k at range i and column j; μ is the overall mean; v_k is the check k effect; and e_{ij} is random error with $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. The assumption was that the estimated residual of each check plot consisted of the spatial pattern and the experimental error. The TPS was implemented with the check residuals to predict spatial patterns as a correction factor across test fields on the progeny-row plot base. The correction factor then was used to adjust yield observations.

For the combined analysis of variance across two years in the Early Generation Tests, the analysis of variance was conducted using the following model with RM as a covariate:

$$y_{ij} = \mu + RM + v_i + y_j + vy_{ij} + e_{ij} \qquad [6]$$

where y_{ij} is the yield or the yld_adj of line i in year j; μ is the overall mean; v_i is the line i effect; y_j is the year j effect; vy_{ij} is the line i and year j interaction effect; and e_{ij} is a random error having a normal distribution $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. Data were analyzed using PROC GLM procedure of SAS version 9.3.2 (SAS Institute,

Cary, NC). yield or yld_adj were analyzed using the same model as described in equation [6] with the PROC MIXED procedure of SAS version 9.3.2 (SAS Institute, Cary, NC) where v and y were the random effects, and RM was used as a covariate. The best linear unbiased prediction (BLUP) of each line in each of the 19 bi-parental populations was predicted based on the two-year data. The performance of each population was the mean performance of the lines within the population.

For individual year analysis of the Early Generation Tests, the following model was assumed with RM as a covariate and the effects of line and block as random effects:

$$y_{ij} = \mu + RM + v_i + b_j + e_{ij} \quad [7]$$

where y_{ij} is the yield or yld_adj of line i at block j; μ is the overall mean; v_i is the line i effect; b_j is the block j effect; and e_{ij} is a random error having a normal distribution $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. The BLUP of each line was estimated by year, and the performance of the population was estimated by year as well using the mean performance of the lines within the population.

In the Confirmation Study, yield data were analyzed across locations using the linear mixed model with RM as a covariate and the effects of lines and locations as random effects:

$$y_{ij} = \mu + RM + v_i + l_j + e_{ij} \quad [8]$$

where y_{ij} is the yield of line i in location j; μ is the overall mean; v_i is the line i effect; l_j is the location j effect; and e_{ij} is a random error having a normal distribution $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. Yield was analyzed using the PROC MIXED procedure of SAS version 9.3.2 (SAS Institute, Cary, NC). The BLUP was estimated for each line. As in the combined two-year analysis, the performance of the

population was the mean performance of all the lines tested within the bi-parental population.

To assess the efficiency of using the TPS model in correcting yield in the Uniformity Study, the coefficient of variation (CVs) and the square root of mean squared deviation (SRMSDs) on the test-set base were compared between the TPS spatial adjustment and non-TPS adjustment. To identify the magnitude in the error reduction, the IRE was calculated as:

$$\mathrm{IRE}_{\mathrm{TPS}} = \frac{(\mathrm{SRMSE}_{\mathrm{non-TPS}} - \mathrm{SRMSE}_{\mathrm{TPS}})}{\mathrm{SRMSE}_{\mathrm{non-TPS}}} \times 100\%, \qquad [9]$$

where $\mathrm{SRMSE}_{\mathrm{non-TPS}}$ is the square root of mean sum of squares for error (SRMSE) from the model without the TPS spatial adjustment, and $\mathrm{SRMSE}_{\mathrm{TPS}}$ is SRMSE from the model with the TPS spatial adjustment, multiplied by100%, to express the value as percentage.

In the Early Generation Tests, $\mathrm{IRE}_{\mathrm{TPS}}$ was calculated also from $\mathrm{SRMSE}_{\mathrm{non-TPS}}$ and $\mathrm{SRMSE}_{\mathrm{TPS}}$ based on the combined two-year data analysis using the model as described in equation [6] for each yield and yld_adj. The Pearson correlation coefficients, between the BLUPs estimated from the Early Generation Tests and that estimated from the Confirmation Study on the basis of the bi-parental population performance, were calculated to measure the efficiency of the TPS model. The rank correlation coefficients, between the BLUPs estimated from the Early Generation Tests and that estimated from the Confirmation Study on the basis of the individual line performance, were calculated to assess effectiveness of selection of the superior lines in the Early Generation Tests after adjustment by the TPS. The Pearson and the rank correlation coefficients were calculated using the PROC CORR procedure of SAS version 9.3.2 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Uniformity Study

Quantification of Non-genetic variation in the Test-set Groups. The results from the Uniformity Study indicated that the spatial variation in the field could be important and might bias the yield estimates when not taken into consideration (Table 2). The two lines used in the Uniformity Study were pure stable commercial lines with proven performance (Lussenden, personal communication, Monsanto Inc. 2012). Yield variations, however, were observed within test-sets suggesting the variation might be non-genetic variation. The CV for yield ranged from 8.6% to 18.4% for MON12, and from 10.5% to 21.6% for MON14 (Table 2). The average of the CV in the test-sets was 11.6% for MON12 and 14.9% for MON14.

For individual test-sets, the SRMSDs for yield were as large as 664.0 kg ha^{-1}, with a mean of 482.2 kg ha^{-1}, and ranged from 339.2 kg ha^{-1} to 664.0 kg ha^{-1} for MON12. For MON14, the SRMSDs were also large, 944.3 kg ha^{-1}, with a mean of 661.0 kg ha^{-1} and range from 446.9 kg ha^{-1} to 944.3 kg ha^{-1} (Table 2). The CV and the SRMSD values indicated that the Uniformity Study had a large component of non-genetic variation within the field on a test-set base. The variations observed among CVs and among SRMSDs in the test-sets were also large, suggesting that the magnitudes of the spatial patterns across the whole test field could be also substantial. These observations agreed with previous results (Duncan, 1969; Tovey et al., 1973). Tovey et al. (1973) reported similar results in sugar yields conducted with sugarcane [*Saccharum officinalis* (L.)], in which plot variations were measured in small test plots. The authors indicated different reasons to explain the presence of non-genetic variation. According to Duncan (1969) and Tovey et al. (1973), potential bias and

variation in yield might be caused from competition effects among neighboring test lines. Variation from non-controlled environmental factors, agricultural practices, and human errors also may contribute to the unexplained variation among plots as mentioned by Wishart and Sanders (1955).

TPS Efficiency in Determining Spatial Effects. The TPS spatial model for field spatial effect adjustment was applied to the yield observations of the Uniformity Study to calculate the CVs and SRMSDs for the yld_adj within test-sets (Table 2). There were reductions observed on both the CVs and SRMSDs. For individual test-sets, the CV for yld_adj ranged from 4.1% to 10.0%, with a mean value of 6.7% for MON12. For Mon 1431 the range was from 6.8% to 14.6%, with a mean of 9.9% for MON14. The mean reduction of CVs from the TPS spatial adjustment was 4.9% (P = 0.002) for MON12, and 5.0% (P = 0.008) for MON14; both highly significant. The SRMSDs for yld_adj ranged from 172.1 kg ha^{-1} to 414.8 kg ha^{-1} with a mean of 281.1 kg ha^{-1} for MON12. For MON14, the SRMSDs for yld_adj ranged from 297.1 kg ha^{-1} to 641.7 kg ha^{-1} with a mean of 436.5 kg ha^{-1}. The mean reduction of SRMSD from the TPS spatial adjustment was 201.1 kg ha^{-1} (P = 0.001) for MON12, and 224.5 kg ha^{-1} (P = 0.011) for MON14, also both significant. The reduction rates in mean CV and mean SRMSD due to the TPS spatial adjustment were 37.1% and 37.2%, respectively. The variation still observed in the yld_adj was assumed to be due to the experimental error.

The results of the analysis of variance for the yield and yld_adj indicated that yld_adj had a significantly smaller mean square for error than yield (Table 3). The IRE_{TPS} was 37.9%, suggesting that the use of the TPS model could improve line comparisons, and also improve the probability of detecting smaller yield differences between lines due to the increase in estimate precision.

Prior to data adjustment by the TPS spatial model, the yield differences between the lines MON12 and MON14 could not be detected, although on average MON14 yielded 242.0 kg ha^{-1} more than MON12. The yield of each of the two lines was not significantly different, due to a large block mean square and large line x block interaction mean square (Table 3), which indicated spatial heterogeneity across blocks and within-blocks. After the TPS spatial adjustment, the mean squares from block and line x block interaction were minimal (P = 0.977, for block effect of zero, and P = 0.987 for line x block interaction effect of zero). The TPS model adjustment effect was reflected on the comparison of the line means. The F-value for line effect was much higher under the yld_adj and highly significant (P = 8.9701E-18). After the TPS adjustment, the analysis was performed with yld_adj, it could be concluded that MON14 yielded 242.0 kg ha^{-1} more than MON12. The superiority of the TPS spatial analysis was evident by the greater genotypic discrimination ability in the test. The results from the Uniformity Study suggested that spatial variation also might be present in the PRYT field tests and that using the TPS spatial model could improve the efficiency of the test. Stinger and Cullis (2000), suggested that since genotypic adjustment for the effects of the relative line position in the field was environmental in nature, the use of the TPS model could lead to improved progeny selection and greater breeding efficiency.

Early Generation Test

Quantification of Spatial Patterns Using Checks in Large Scale Early Generation Tests. In the 2010 and 2012 Early Generation Tests, a trend in yield variation was observed; i.e. the yield of lines increased from the upper to the lower area of the test field

(Fig. 1). Observed yields could result from the combination of line genetic merit, field spatial pattern, and experimental error (Bernardo, 2003).

The field spatial patterns in the Early Generation Tests were evaluated using a sub-set of data only containing the three common checks. The analysis of residuals from the linear model with check as a fixed effect revealed that the yield distribution of the checks was affected by their spatial location within the field. In the 2010 and the 2012 field tests, the residuals for the checks placed in the upper area of the field were smaller compared to the residual of the checks in the lower area.

To quantify the field spatial patterns in yield separately for each year, 2010 and 2012, the spatial effects across the test fields were predicted based on the TPS model used with check residuals (Fig. 2). In both years, the predicted spatial effects on yield were smaller in the upper half area and larger in the lower half area. In 2010, the spatial effect could be as small as -2473.8 kg ha^{-1} in the upper half area, and as large as 1820.7 kg ha^{-1} in the lower half area. In 2012, the spatial effect could be as small as -1339.0 kg ha^{-1} in the upper half area, and as large as 1306.8 kg ha^{-1} in the lower half area. The patterns in estimates of spatial effects of the TPS model (Fig. 2) were similar to the field spatial trends in yield (Fig. 1), and in check residuals (Fig. 2). The observations indicated that field spatial trends due to heterogeneous environments could be identified and removed by the use of the TPS model. In 2012, the severe drought in the Midwest of North America during the pod filling season (Table 1) also might have interacted and affected the field spatial trend that was observed for the year.

TPS Efficiency in Determining Spatial Effects. Data with and without spatial adjustment (yld_adj and yield) in 2010 and in 2012 Early Generation Tests were fitted into the general linear model for the analysis of variance using the model in equation [6]. The mean square for error from the analysis of yld_adj was smaller than that of the yield (Table 4), and the $IRE_{TPS}$was 40.4%. The value was similar to the IRE observed in the Uniformity Study (Table 3). The results of the Early Generation Tests indicated that the TPS spatial model was effective in capturing the spatial patterns in a large field, similarly as it was effective at a smaller scale in the Uniformity Study. The reduction of the error variance also indicated that the TPS model could improve line comparisons, allowing detection of small differences between lines by reducing the LSD. Robbins et al. (2012) describing work conducted with un-replicated maize trials also concluded that the TPS spatial model could effectively reduce the error variance by accounting for spatial variations.

Confirmation Study

Selection Efficiency Improvement Using the TPS Spatial Model. In a soybean breeding program the rows of the un-replicated PYRT test would be bulk-harvested and the superior high-yielding genotypes would be selected for planting larger plots at multiple locations during the following year. In the study reported here, all genotypes tested in the Early Generation Test were planted in 2011 at each of four locations in Iowa, in replicated field tests with two-row plots for every genotype. All lines from the Early Generation Tests were planted in the Confirmation study to be able to compare the individual line yield performance in each of the tests planted.

The first yield performance comparison was made by calculating the Pearson correlation coefficients between the mean performances of the individual bi-parental populations, in the individual years and combined over two years of the Early Generation Tests for the observed yield and the yld_adj, and that of the same bi-parental populations evaluated in the Confirmation Study (Table 5). In the data-set from the year 2010, the correlation coefficient of the estimated yield increased from 0.43 (P = 0.065) to 0.54 (P = 0.018) after the use of the TPS spatial adjustment. This represented a positive change of 0.11 points in the correlation due to the use of the TPS model. In the year 2012, similar results were observed. The correlation increased from 0.24 (P = 0.324) to 0.57 (P = 0.011), an improvement of 0.33 points in the correlation value due to the use of the TPS adjustment.

Additional improvement in the size of the correlation value also was observed when the combined years of the data-set of the Early Generation Tests were used (Table 5). The correlation coefficient with the TPS adjustment was 0.61 (P = 0.006) and without the TPS adjustment was 0.40 (P = 0.094). The improvement in the correlation value due to the TPS adjustment was of 0.21 points. Similar results also were reported by Edmé et al. (2007) in the sugarcane work.

To assess if the TPS model adjustment used in the Early Generation Tests would have an effect on identifying individual line performance of yield, a 1% selection intensity was applied to the lines tested in the Early Generation Tests, both on the basis of actual yield and the yld_adj. The lines were ranked according to yield as evaluated on the Early Generation Test from 1 to 26 (data not shown). The ranking of the selected lines then was compared to the rank of the same lines evaluated in the Confirmation Study. The selection

was practiced across populations by individual years, 2010 and 2012, and also for the yield averaged over the two years. The rank correlations were calculated for each of the selection environments and the yield selection criteria, actual yield and adjusted yield. None of the rank correlations among the studies were significant indicating that the line ranking at each test was different. When the selections were made using the 2010 yield data of the Early Generation Test, only four of the selected lines were among the superior 26 genotypes in the Confirmation Study. None of the lines selected in the Early Generation test in 2012 could be found among the 26 best lines of the Confirmation Study. When selections were based on the combined yield data of 2010 and 2012, none of the selected lines were among the best 1% of the lines identified in the Confirmation Study. These observations suggest that although the TPS spatial adjustment was effective in controlling spatial variation, the adjustment may still not be enough to reflect an improvement in the prediction of line performance of the Early Generation tests on the basis of the progeny-row.

CONCLUSIONS

This research was conducted to determine if the TPS model could effectively remove variation due to non-genetic spatial trends from the early generation tests. The underlying assumption was that the error term would be reduced by the adjustment, thus allowing a more precise comparison among yield of lines that were genetically diverse. The results of two of the studies, namely the Uniformity Study and the Early Generation Test, indicated that the TPS model was an effective tool for removing non-genetic spatial variation in soybean PRYT field tests. The use of the TPS adjustment in both studies effectively reduced

the coefficient of variation and the square root of the mean square deviation in each test. Additionally, the efficiency in hypothesis testing was increased as determined by the IRE values.

The third study, the Confirmation Study, was designed to test if the removal of the non-genetic variation in field tests done by adjusting yield using the TPS spatial model would be reflected in an increase in the efficiency of line selection. If the lines selected after use of the TPS spatial adjustment for yield, also would have been among the superior lines in the Confirmation Study, this would have been an indication of the effectiveness of the TPS spatial adjustment to increase the precision of the yield estimation at the early stages of breeding. This was not observed. The results suggested that use of the TPS model as performed in this research would not increase selection precision of lines at the early generations. The TPS model adjustment did increase the Pearson correlation coefficients among the yield results from the Early Generation yield tests and the yield results from the Confirmation Study on the basis of bi-parental population performance. The positive change in the correlation values, however, was not reflected in the individual line performance *per se.* When a selection intensity of 1% was used to identify the highest yielding lines in the Early Generation Tests, and their performance in the Confirmation Study was determined, the number of superior lines in both studies was equal to six or less regardless of the TPS adjustment or not. These results indicated that under the conditions of this research, there was no improvement on the selection efficiency after using the TPS spatial adjustment.

A factor to be considered in interpreting the results, however, is the number of checks used to model spatial variation. In the research three checks replicated throughout

the field were used. The total number of lines evaluated in a test-set of the Early Generation Test was 45, and the total number of checks was three. The ratio of number of lines to checks was 15 to 1. It might be that the number of checks might have been too small compared to the number of lines evaluated. This ratio, in turn, might have caused an underestimation of the field spatial variation, which could have contributed to the lack of prediction of the Early Generation Tests compared to line performance evaluated in the Confirmation Study. Research is in progress to determine if an increase in the number of checks in the Early Generation Tests could increase the selection efficiency at early stages of soybean breeding.

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Table 1 Weather information for the research location of Monsanto Inc. in Central IA that was used in the conduct of the 2010 and 2012 Early Generation Tests.

Weather	Year	April	May	June	July	August	Sept.
Rainfall (mm)	2010	107.4	96.1	304.4	134.9	313.4	211.1
	2012	107.2	57.5	71.9	56.6	67.3	54.6
	1997 to 2011†	97.3	141.1	137.3	110.9	118.7	80.7
Temperature‡ (°C)	2010	12.9	15.7	22.0	24.0	24.0	17.7
	2012	11.5	19.2	22.4	26.7	21.8	17.1
	1997 to 2011†	9.9	15.8	20.9	23.6	22.2	17.7

† Averages of rainfalls and daily temperatures from 1997 to 2011

‡ Daily temperature was the mean of daily maximum and minimum temperatures

Note: The soil at the research location in Central IA is sandy clay loam. Sowing date was May 5 in 2010; sowing date was May 10 in 2012.

Table 2 Variation within test-set in the yield observations recorded in the Uniformity Study, without the TPS spatial adjustment (yield), and with the TPS spatial adjustment (yld_adj).

Test-set Code	yield			yld_adj		
	Mean (kg ha^{-1})	SRMSD† (kg ha^{-1})	CV† (%)	Mean (kg ha^{-1})	SRMSD† (kg ha^{-1})	CV† (%)
			MON12‡			
1	3601.8	664.0	18.4	4169.6	414.8	9.9
2	4377.8	380.5	8.7	4184.4	254.8	6.1
3	4352.4	444.9	10.2	4161.6	236.5	5.7
4	4442.4	584.6	13.2	4168.6	354.0	8.5
5	3646.1	352.3	9.7	4177.8	187.6	4.5
6	3943.2	339.2	8.6	4184.4	172.1	4.1
7	4319.1	466.3	10.8	4162.0	305.6	7.3
8	4732.2	625.7	13.2	4190.1	323.5	7.7
Grand mean	4176.9	482.2	11.6	4174.8	281.1	6.7
			MON14‡			
1	3997.2	466.7	11.7	4428.6	323.1	7.3
2	4254.9	589.5	13.9	4419.2	390.2	8.8
3	4599.4	660.1	14.4	4426.2	474.6	10.7
4	4731.9	811.6	17.2	4417.2	473.1	10.7
5	4182.9	904.3	21.6	4426.7	570.5	12.9
6	4393.8	464.4	10.6	4404.3	321.6	7.3
7	4256.6	446.9	10.5	4388.8	297.1	6.8
8	4888.9	944.3	19.3	4411.0	641.7	14.5
Grand mean	4413.2	661.0	14.9	4415.3	436.5	9.9

† CV = coefficient of variation; SRMSD = square root of the mean square deviation.

‡ MON12 and MON14: soybean commercial cultivars released by Monsanto Inc. in 2010 and 2012, respectively.

Table 3 Analysis of variance of the Uniformity Study based on yield observations without the TPS spatial adjustment (yield) and with the TPS spatial adjustment (yld_adj).

Source	DF	Mean square (kg ha^{-1})	F-value	P-value†
		yield		
Line (v)	1	10724151.0***	28.91	1.01E-07
Block (b)	3	23375835.7***	63.01	2.18E-36
v x b	3	1263073.0*	3.40	0.017
Error	760	370992.2		
		yld_adj		
Line (v)	1	11100877.7***	77.47	8.97E-18
Block (b)	3	9673.7	0.07	0.977
v x b	3	6631.0	0.05	0.987
Error	760	143283.9		
		IRE_{TPS}‡ = 37.9%		

*, **, *** F test significant at P = 0.05, P = 0.01, and P = 0.001, respectively.

† 1.01E-07 = 1.01 x 10^{-7}; 2.18E-36 = 2.18 x 10^{-36}; 8.97E-18 = 8.97 x 10^{-18}.

‡ $IRE_{TPS} = ((SRMSE_{Yield} - SRMSE_{yld\_adj}))/SRMSE_{yield} \times 100\%$.

Table 4 Analysis of variance of the Early Generation Test combined over years with relative maturity (RM) as a covariate based on yield observations without the TPS spatial adjustment (yield) and with the TPS spatial adjustment (yld_adj).

Source	DF	Mean square (kg ha^{-1})	F-value	P-value
		yield		
RM	1	485050.0	0.87	0.350
Year (y)	1	582775396.0***	2745.43	<.0001
Line (v)	2567	1266547.0***	7.42	<.0001
y x v	2567	544476.0***	3.85	<.0001
Error	1199	566068.0		
		yld_adj		
RM	1	176049.0	0.86	0.355
Year (y)	1	552382770.0***	1029.52	<.0001
Line (v)	2567	1492321.0***	2.24	<.0001
y x v	2567	775079.0	0.96	0.786
Error	1199	201201.0		
		IRE_{TPS} † = 40.4%		

*, **, *** F test significant at P = 0.05, P = 0.01, and P = 0.001, respectively.

† $IRE_{TPS} = ((SRMSE_{yield} - SRMSE_{yld\_adj}))/SRMSE_{yield} \times 100\%$.

Table 5 Analysis on the basis of bi-parental population performance for Pearson correlation coefficients (top) and P-values (bottom) for significance between the Confirmation Study, and the Early Generation Tests for two years and by year with yield observations without the TPS spatial adjustment (yield) and with the TPS spatial adjustment (yld_adj), respectively.

Population performance	BLUP1012 _tps†	BLUP1012 _un‡	BLUP10 _tps§	BLUP10 _un¶	BLUP12 _tps#	BLUP12 _un††
BLUP_true¥	0.61 0.006**	0.40 0.094^{ns}	0.54 0.018*	0.43 0.065^{ns}	0.57 0.011*	0.24 0.324^{ns}

Note: BLUP stands for the best linear unbiased prediction for individual populations calculated by the mean of the individual line BLUPs within population.

*, ** F test significant at P = 0.05 and P = 0.01, respectively; ns F test non significant at P = 0.05.

† Population BLUPs were estimated based on yld_adj from two-year Early Generation Tests.

‡ Population BLUPs were estimated based on yield from two-year Early Generation Tests.

§ Population BLUPs were estimated based on yld_adj from the Early Generation Test in 2010.

¶ Population BLUPs were estimated based on yield from the Early Generation Test in 2010.

Population BLUPs were estimated based on yld_adj from the Early Generation Test in 2012.

†† Population BLUPs were estimated based on yield from the Early Generation Test in 2012.

¥ Population BLUPs were estimated based yield from the Confirmation Study.

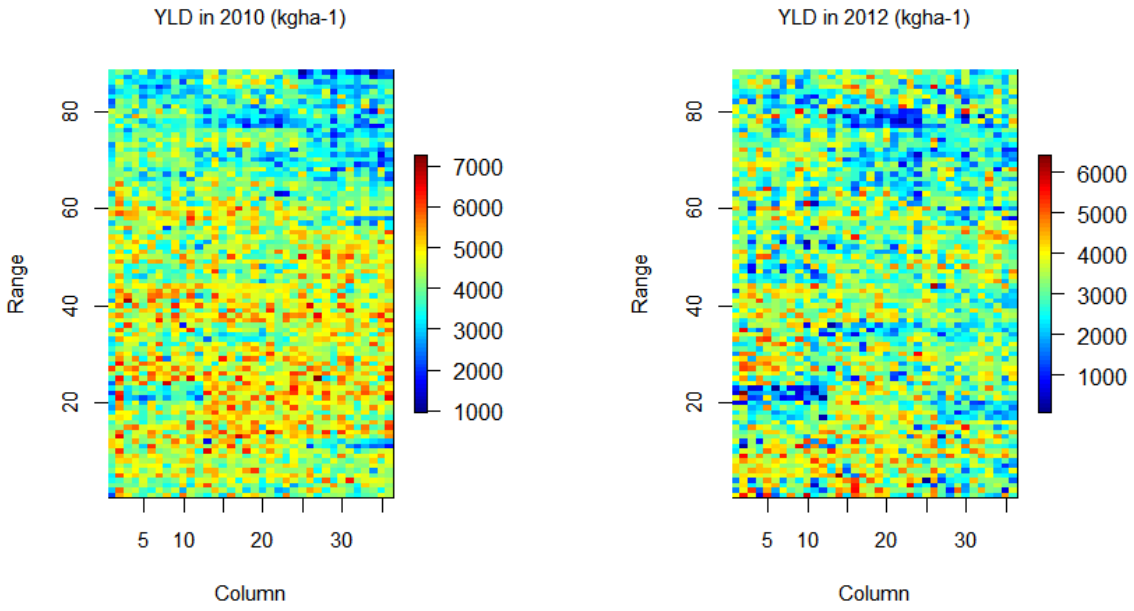

Fig. 1 Heat map for yield observations without the TPS spatial model adjustment (yield) of the Early Generation Tests in 2010 (left) and 2012 (right), respectively.

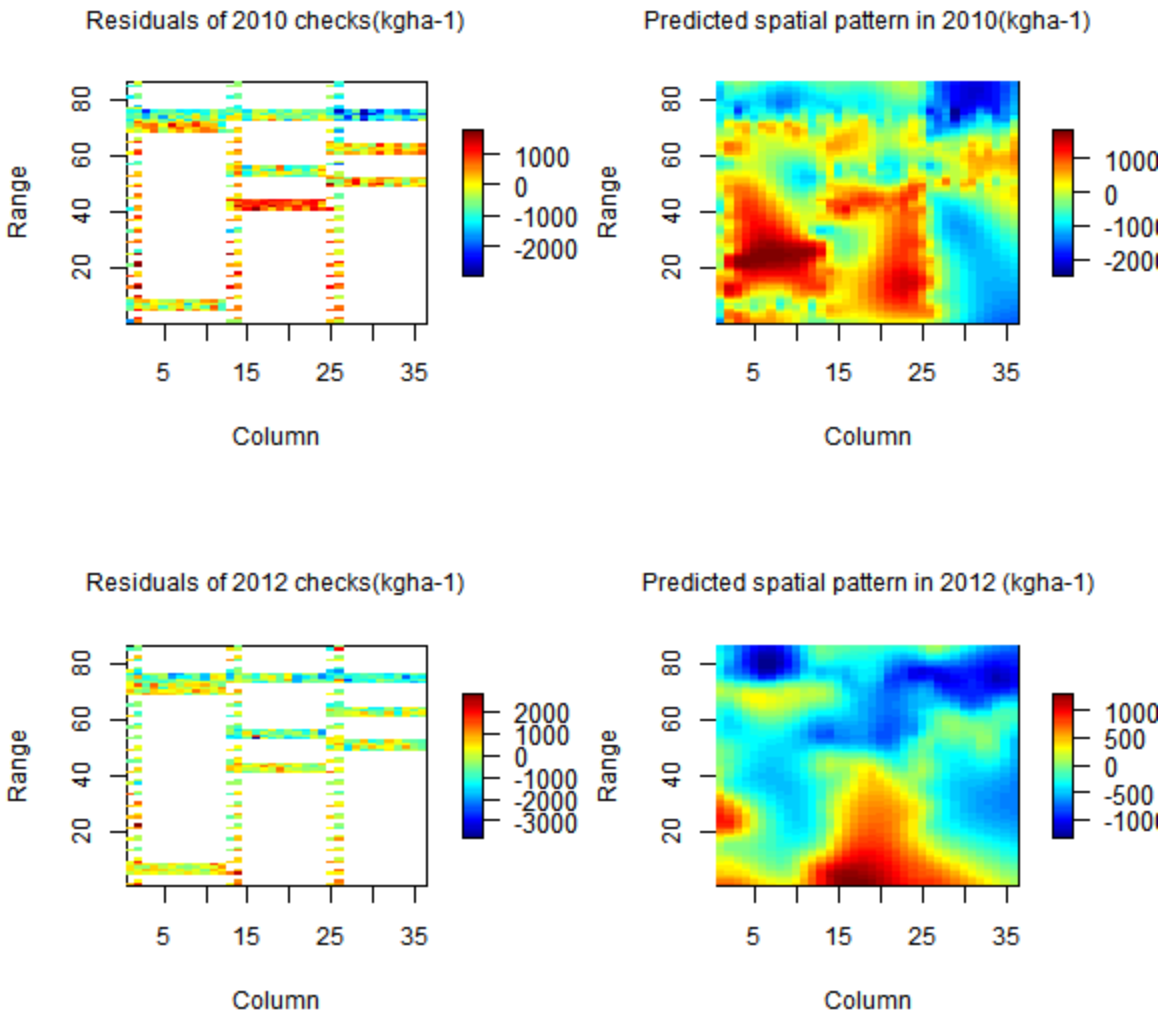

Fig. 2 Heat maps of check residuals and the predicted spatial effects. Heat map of check residuals estimated from the linear model with checks as fixed effects based on the check plot yield observations from the Early Generation Test in 2010 (top/left), and the heat map of the predicted spatial patterns using the TPS spatial model based on the check residuals from 2010 Early Generation Test (top/right); the heat map of check residuals estimated from the linear model with checks as fixed effects based on the check plot yield observations from the Early Generation Test in 2012 (bottom/left), and the heat map of the predicted spatial patterns using the TPS spatial model based on the check residuals from 2012 Early Generation Test (bottom/right).

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CHAPTER 3. PREDICTABILITY STUDY OF SOYBEAN LINE PERFORMANCE BASED ON MULTIPLE YEAR AND LOCATION TRIALS

M. Sun[1*], S. A. Goggi[2], K. Matson[1], R. G. Palmer[2], K. Moore[2], and S. R. Cianzio[2*]

[1] Monsanto Inc., St. Louis, Missouri, USA

[2] Department of Agronomy, Iowa State University, Ames, Iowa 50011-1010, USA

Address correspondence to M. Sun at 3302 SE Convenience Blvd, Ankeny, IA 50021, USA. E-mail: msun0203@yahoo.com

Address correspondence to Dr. Cianzio at 2107 Agron. Hall, Ames, IA 50011-1010, USA. E-mail: scianzio@iastate.edu

ABSTRACT

The multiple environment trial is conducted as a means to predict the true genetic potential of test lines, and then select true genetic superior lines for commercialization in soybean breeding. In multiple environment trial, line x environment interaction is omnipresent, and significantly impacts the effort for accurate and stable prediction of the line performance. The knowledge of the compositions of line x environment interaction (GxE) effect can and should be used to design optimal multiple year and location trials for accurate and stable prediction of line performance with the given resource. The objectives of this research were to i) quantify the effects of line x year interaction (GxY), line x location interaction (GxL), and three-way interaction of line x year x location (GxYxL) using a wide arrange of years and locations to evaluate elite soybean lines; and ii) evaluate prediction efficiency of single-year multiple-location yield trials. This study was conducted using multiple year and location trials (MYLTs) for soybean of each of three maturity groups,

relative maturity (RM) groups of RM 1.0 to 1.9, RM 2.0 to 2.9, and RM 3.0 to 3.9. The results indicated that i) GxE is omnipresent with varied percentage of the explained variation contribution among total observed yield variation, and the predominant source of GxE is GxYxL; ii) the significant presence of GxE and year x location interaction (YxL) warrants multiple-year and multiple-location trials for sufficient predictability of line performance in the following year. However, two-year multiple-location trial should be sufficient to capture the soybean top-yielding lines. If a trial with relatively low presence of GxE, one-year multiple-location trials should be sufficient to capture the soybean top-yielding lines.

Keywords Genetic gain, best linear unbiased prediction, soybean breeding, genotype x environment interaction.

Abbreviations and Nomenclatures (MYLT) multiple year and location trial; (YxL) year x location interaction; (GxE) line x environment interaction; (GxY) line x year interaction; (GxL) line x environment interaction; (GxYxL) line x year x location three-way interaction; (IQR) interval quartile range; (BLUP) best linear unbiased prediction for yield; (MAT) maturity date; (RM) relative maturity; (ANOVA) analysis of variance; (REML) restrict maximum likelihood. (r) Pearson product-moment correlation coefficient; (ρ) Spearman's rank correlation coefficient.

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INTRODUCTION

In breeding and improvement of crops, genetic gain per-year determined for a target trait is the primary measure of the success of the program. Among many sources of variation that could determine the success of the breeding program, genotype x environment interaction (GxE) present in multiple environment trials is one of the major factors that impact genetic gain (Fehr, 1993). From a statistical point of view, GxE is defined as change in rank of the line performances across locations, namely crossover GxE, or change in the magnitude of difference of line performances across test locations, namely non-crossover GxE (Haldance, 1947; Mather and Caligari, 1976; Gregorius and Namkoong, 1986; Baker, 1988, 1996). To achieve high genetic gain for low heritability traits, such as yield, plant breeders designed testing and selection strategies that might help to account for GxE effect (Fehr, 1993). One approach is the use of the multiple year and location trials (MYLT) that will allow measuring GxE effects and more precisely evaluate line performance.

From theoretical studies in the MYLT, effect of GxE could be partitioned into genotype x location interaction (GxL), genotype x year interaction (GxY), and three-way interaction of genotype x year x location (GxYxL) (Comstock and Moll, 1963; Annicchiarico and Perenzin, 1994; Yan and Rajcan, 2003). To estimate GxL effects, trials are conducted in multiple locations during a single year. Similarly, to estimate GxY effects, multiple-year trials are conducted, and by having multiple years and locations, then effects of GxYxL also are estimated (Yan and Rajcan, 2003). It is assumed that the MYLT could provide more accurate estimates of true genetic values of the test lines, and more precisely predict the line performance in years and locations in which the lines will be planted in the future (Yan

and Rajcan, 2003). The published information comparing the efficiency of one-year multiple-location trials *vs* MYLTs is limited, and results were contradictory depending on the crop species and within crops also depending on maturity group of the test lines (Cross and Helm, 1986; Gellner, 1989; Bowman, 1998; Yan and Rajcan, 2003). Based on the analysis of combined 10-year data of the Ontario Soybean Variety Trials in four locations from 1991 to 2000, Yan and Rajcan (2003) could not partition GxE effect into GxY, GxL, and GxYxL limited by the attributes of the data. However, based on the Pearson product-moment correlation coefficient (r) between line performance in one year estimated using one-year multiple-location trials and that estimated in the next year multiple-location trials, they concluded that one-year multiple-location trial was sufficient to predict soybean line performance in the next year. Using a balanced sub-set of data with barley (*Hordeum vulgare* L.), maize (*Zeu mays* L.), cotton (*Gossypium hirsutum* L.), oat (*Avena sativa* L.), soybean (*Glycine max* (L.) Merr.), and wheat (*Triticum aestivum* L.) from the North Carolina Official Variety Trials, Bowman (1998) found that GxL was not present in all six crops, GxY occurred with barley and cotton, and GxYxL was important with barley, wheat, and soybean at RM group VI. Based on the probability of the turnover for the top-yielding lines, he concluded that all six crops needed two-year multiple-location trials to ensure the sufficient prediction efficiency for top-yielding lines in the next year, except mid-season corn hybrids which only needed one-year multiple-location trials. Based on the analysis of spring wheat and oat data from the South Dakota Cultivar Test from 1972 to 1987, Geller (1989) concluded that one-year multiple-location trials were equally good for prediction of the next year performance as that based on previous two- or three-year trials.

The objectives of this research were therefore to i) quantify the effects of GxY, GxL, and the three-way interaction of GxYxL using a wide arrange of years and locations to evaluate elite soybean lines; and ii) evaluate prediction efficiency of single-year multiple-location yield trials. The study was conducted using MYLTs for soybean of each of three maturity groups, relative maturity (RM) groups of RM 1.0 to 1.9, RM 2.0 to 2.9, and RM 3.0 to 3.9.

MATERIALS AND METHODS

The study consisted of three separate trials each corresponding to a different maturity group, trial 1 identified as GXE15 included lines with relative maturity (RM) from 1.0 to 1.9; trial 2 identified as GXE25 included lines with RM from 2.0 to 2.9; and the third trial identified as GXE35 covered lines with RM from 3.0 to RM 3.9. Each of the three trials was planted in the following four years 2009, 2010, 2011, and 2013, and in each year the same group of 20 lines in each trial was always included. Within each of the four years, GXE15 were planted at the same 16 locations, GXE25 at the same 23 locations, and GXE35 at the same 19 locations. For planting the trials, the lines were selected based on the 2008 multiple-location trials on the basis of the following criteria i) the lines were of highest yielding potential in each maturity group, and ii) the lines were diverse genetically according to pedigree information. The seed sources of the lines for the trials were from the previous-year yield trials.

The locations were selected from the Monsanto Inc. test network sites that were diverse environmentally mainly in terms of soil types and rainfall patterns. The soil types of the test locations for GXE15 and GEX35 trials ranged from clay loam to sandy loam, and

from clay loam to silt loam for GxE25. Across years, the planting dates of GXE15 were from May 7 to June 9, depending on the year and locations. The planting dates of GXE25 were from May 4 to June 18; and from May 4 to June 11 for GXE35, also depending on location and year.

The 20 elite lines at each trial were evaluated using a randomized complete block design (RCBD) with two replications per location. Within replications, the 20 lines were distributed randomly according to a grid four ranges deep and 10 columns wide. The plots were two-row plots, 3.7 m in length, with 0.8 m between rows resulting in a sowing population of 30 seeds m^{-1}.

Each trial was planted and harvested mechanically, utilizing computer technology to automatically record grain harvest and grain moisture. The agronomic data recorded in each individual plot were maturity date (MAT) at reproductive stage R7 (Fehr et al., 1971), and grain yield measured in kg ha^{-1} at reproductive stage R8 (Fehr et al., 1971) with a moisture correction factor to express yield at 13.5% moisture content. Each plot at each trial was harvested in bulk.

Statistical Analysis

The assumptions of normality and homogeneity of variance in the error term of yield data from each of the three trials were evaluated before any parametric statistical analysis was conducted. In all trials, the yield data were continuous with near normal distribution based on the test of QQPLOT in the PROC UNIVARIATE procedure of SAS version 9.3.2 (SAS Institute, Cary, NC).

Box-plots based on the interval quartile range (IQR) method (Tukey, 1977) were implemented for outlier identification. For each individual location, IQR was calculated across the two replications. On the basis of the research conducted by Tukey (1977) and Eo et al. (2012), an observed yield value is treated as an outlier when

$$y < q1 - 1.5*iqr \text{ or } y > q3 + 1.5*iqr \quad [1]$$

where y is the observed yield; q1 was the 25% sample quartile, q3 was the 75% sample quartile, and IQR was the difference between q3 and q1, for a given data set. An outlier is, therefore, treated as a missing value, otherwise, to avoid distorting the statistical inference. The yield data from each of the three trials were analyzed using SAS 9.2.3 statistical package (SAS Institute, Cary, NC).

Across years and locations, yield data of each trial were analyzed using the linear mixed model considering effects of year, location, and line as random effects:

$$Y_{ijk} = \mu + G_i + Y_j + L_k + GxY_{ij} + YxL_{jk} + GxL_{ik} + GxYxL_{ijk} + e_{ijk} \quad [2]$$

where Y_{ijk} is the yield of line i in year j at location k; μ is the overall mean; G_i is the line i effect; Y_j is the year j effect; L_k is the location k effect; GxY_{ij} is the line i and year j two-way interaction effect; YxL_{jk} is the year j and location k two-way interaction effect; GxL_{ik} is the line i and location k two-way interaction effect; $GxYxL_{ijk}$ is the line i and year j and location k three-way interaction effect; and e_{ijk} is a random error having a normal distribution $e_{ijk} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2.

Data were analyzed using the PROC MIXED procedure of SAS version 9.3.2 (SAS Institute, Cary, NC) for the variance components, and the BLUPs of lines based on data across years and locations. To estimate the percentage of variation that each factor contributed to the observed yield variation, yield data also were analyzed using the same

model as described in equation [2] with the PROC GLM procedure of SAS version 9.3.2 (SAS Institute, Cary, NC) where Y, V, and L were considered as fixed effects for analysis of variance.

For the analysis using two-year and three-year data, the same PROC MIXED procedure of SAS version 9.3.2 (SAS Institute, Cary, NC) was used with the same model described in equation [2] to estimate the BLUPs of test lines.

For the analysis within years across locations, yield data were analyzed using the linear mixed model with effects of location, line as random effects:

$$Y_{ik} = \mu + G_i + L_k + GxL_{ik} + e_{ik} \quad [3]$$

where Y_{ik} is the yield of line i at location k; μ is the overall mean; G_i is the line i effect; L_k is the location k effect; GxL_{ik} is the line i and location k two-way interaction effect; and e_{ik} is a random error having a normal distribution $e_{ik} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. Data were analyzed using the PROC MIXED procedure of SAS version 9.3.2 (SAS Institute, Cary, NC) for the BLUPs of the lines based on the data across locations within years.

To assess the significance of the variance components greater than zero based on the data across four years and locations, the COVTEST function was used, which was based on Z-test, in the PROC MIXED procedure of SAS version 9.3.2 (SAS Institute, Cary, NC). The yield predictabilities of the trials, by-year, combined two-year, and combined three-year, were measured by the Spearman rank correlation coefficients (ρ) between the BLUPs estimated under each of the scenarios and that estimated based on another year multiple location trial. The Spearman's ρs were calculated using the PROC CORR procedure of SAS version 9.3.2 (SAS Institute, Cary, NC).

It is widely accepted that line BLUP value estimated from multiple year and location trials, based on the PROC MIXED procedure of SAS version 9.3.2 (SAS Institute, Cary, NC), is considered as the true genetic value of the line (Cervantes-Matrinez et al., 2001, 2002). The more years of data used, the better the estimated BLUP value as the true genetic value. In this research, the selections of top-yielding lines based on the combined four-year multiple location trials were considered as true genetic superior lines. To assess if, or not, the predictability from the combined two-year trials or the combined three-year trials had significant gain compared to that from one-year multiple location trials, a 25% selection intensity was applied to the lines tested under each of the test scenarios. Then, the number of the overlapping lines selected with the ones based on the combined four-year multiple location trials was used to measure the predictability of the trials.

RESULTS AND DISCUSSION

Variance components of the observed yield variation and dissection of the variance of line by environment interaction. The results from analysis of variance (ANOVA), by year and trial, indicated that the contributions of variables to the observed yield variation were significantly different in multiple-location trials within a year (Table 1). The predominant source of the variation was the main effect of location. Across four years and three trials, the percentage of the variation explained by location main effect ranged from 57.2% in GXE35 in 2009 to 84.9% in GXE15 in 2013, with a mean of 72.3% and a square root of mean square deviation (SRMSD) of 7.9%. The main effect of line only explained a small percentage of the variation which ranged from 2.2% in GX15 in 2010 and 2013 to 11.4% in GXE35 in 2013, with a mean of 5.4% and a SRMSD of 2.6%. Compared to line main effect,

line by location interaction explained a larger percentage of the variation which ranged from 7.2% in GXE15 in 2013 to 21.0% in GXE35 in year 2009, with a mean of 13.6% and a SRMSD of 4.1%. The line main effect in GXE15 in 2010 was not significant larger than zero at P = 0.05, however, all the other effects across four years and three trials were significant larger than zero. Particularly, the effects of GxL across years and trials were highly significant with P values ranged from 6.0×10^{-3} in GXE15 in 2009 to 2.07×10^{-9} in GXE15 in 2010 with a mean P value of 9.17×10^{-4}. These observations agreed with the statement (Fehr, 1993): "…… *One cultivar may have the highest yield in some environments and a second cultivar may excel in others*. ……". The change of ranks across test locations is one of the two sources of line by location interaction in multiple-location yield trials (Haldance, 1947; Mather and Caligari, 1976; Gregorius and Namkoong, 1986; Baker, 1988, 1996; Fehr, 1993). The significance of line by location interaction effect justifies the multiple-location trials for yield, which is designed not only for increasing the accuracy of the comparison of line mean performance, but most importantly, for the identification of GxE, and consequently, identifying the regions that fits the line best.

Based on within-year analysis of the data from the Ontario Soybean Variety Trials with four locations in the 2800 Crop Heat Unit area from 1991 to 2000, Yan and Rajcan (2003) reported the result that location main effect was the main source of the observed yield variation, which was similar to this study. It explained 59.3% of the yield variation on the mean base over 10 years. However, they found that line main effect explained a much larger percentage of 18.2% on the mean base with a much larger range of 4.6% to 30.8%, compared to this study with a mean of 5.4% which ranged from 2.2% to 11.4% for the variation contribution. Yan and Rajcan (2003) also could not partition the variance

contribution from line by location (or environment) interaction. The main reasons for the results from Yan and Rajcan analysis might be i), the line performances were estimated based only on four locations, and the small number of the test locations might bias the estimations. ii), the field lay-out within test locations was a lattice design (before 1998) or a nearest neighbor design (1998 and later) with four replications, but, the mean yields of the four replications within locations were used for the analysis.

In this study, we had yield data across four years and multiple locations. The observed yield variations were partitioned into variances of main effects of year, location, and line, two-way interactions of YxL, GxY, and GxL, and three-way interaction of GxYxL, where the sum of the variances from GxY, GxL, and GxYxL formed the sum of variance of GxE (Table 2). The variance estimations were conducted using the PROC MIXED procedure of SAS version 9.3.2 (SAS Institute, Cary, NC) with a variance estimation method of restrict maximum likelihood (REML) (Patterson and Thompson, 1971), where the low boundary of zero was applied to all variance components in the model (Kiernan et al., 2012). The zero estimation of variance component for year main effect in all three trials reflected that there was not enough variation in yield to attribute variation of the year as a random effect controlling all other variables in the model (Kiernan et al., 2012). The high percentage of the variation explained by YxL in each of three trials, which was 38.2% in GXE15, 46.3% in GXE25, and 42.2% in GXE35, indicated that the location attributes, reflected by the mean yield performance of 20 test lines in each trial, were significantly different across four years, which were confirmed by the P-values of YxL for significant test in analysis of variance components. The regression plots, by year, between location and the mean yield of the location showed that the types of YxL effects in all three trials were crossover

interaction (Fig. 1). The significant crossover YxL effect warrants the MYLT for accurate and stable line performance prediction and selection. Similar results were reported by Yan and Rajcan (2003) based on analysis of the combined 10-year data of the Ontario Soybean Variety Trials in four locations from 1991 to 2000, where they found that YxL explained 55.4% of the observed yield variation.

Compared to YxL, GxE explained a smaller percentage of the observed yield variation with 13.2% in GXE15, 12.3% in GXE25, and 17.2% in GXE35. However, all three components of GxE in three trials were highly significant with a P-value less than 0.01, except GxL in GXE35 (P = 0.101) (Table 2). Particularly, the effect of GxYxL was significant with P-values of $1.02X10^{-11}$, $1.53X10^{-8}$, and $4.08X10^{-11}$ in GXE15, GXE25, and GXE35, respectively. This explained 61.4% of GxE variation in GXE15, 55.3% in GXE25, and 60.5% in GXE35. The results indicated that GxYxL was a predominant source within the variation of GxE. Limited by the attribute of the data, which had 83.3% missing rate, and the mean yield across replications was applied before fitting linear mixed model for varaince estimation, Yan and Rajcan (2003) confounded the variance of GxE with residual variance.

The significant presence of YxL and GxE which was represented by the significance of GxY, GxL, and GxYxL in this study across three trials indicated that the MYLT was essential to ensure the accurate prediction and selection of the line-yield performance. In the MYLT, YxL and GxE could be accounted properly. Similar conclusions were drawn by Bowman (1998), and Yan and Rajcan (2003). However, to achieve the high genetic gain, the time of a breeding cycle is one of the main factors (Fehr, 1993). From statistical analysis, multiple-year trials are required given the number of the testing locations within

a year. The questions are i) how well do one-year multiple-location trials predict line performances in another year? ii) how many years are adequate for the MYLT?

The predictabilities of one-year multiple-location trials, two-year multiple-location trials, and three-year multiple-location trials measured Spearman's ρs The line true genetic performances were estimated as BLUPs by the linear mixed model [3] for one-year multiple-location trials, and by the linear mixed model [2] for two-year/three-year multiple-location trials for trials of GXE15, GXE25, and GXE35, separately.

For the predictability of one-year *vs* one-year, the Spearman's ρ between the BLUPs estimated using one-year multiple-location data and that estimated using another year within trials was used (Table 3). Because year was considered as random effect, the pair of the years had no order issue. Therefore, across four years within each trial, there were six distinct pairs of one-year *vs* one-year comparisons. The results indicated that the mean predictability of one-year *vs* one-year varied among the three trials with ρ = 0.42 in GXE15, ρ = 0.70 in GXE25, and ρ = 0.66 in GXE35. The results might be consistent with the ones from ANOVA based on the combined four-year multiple-location yield data (Table 2). In GXE15, the variance of line was very small with 1.5% explanation of the yield variation, but, the variance of GxYxL was relatively large compared to GXE25. Both factors together might have caused the smallest mean ρ of one-year *vs* one-year with a ρ = 0.42 in GXE15 among three trials. GXE25 had the highest mean ρ of one-year *vs* one-year with a ρ = 0.70 among three trials, although it had relatively smaller percentage of the variance explained by line main effect compared to that in GXE35, but, it had the smallest percentage explained by GxYxL with 6.8% among three trials. The conclusion in this study regarding the predictabilities of one-year multiple-location trials varied depending on the attributes of

the trials reflected in the variance components from G and GxYxL. Based on the analysis of Pearson's r between line performance in one year and that in the following year using 10 year trials, Yan and Rajcan (2003) found that the r ranged from 0.41 to 0.71 with a mean of 0.54, which was consistent with the conclusion from this study.

For the predictability of two-year *vs* one-year within trials, the ρ between the BLUPs, estimated using two-year multiple-location data and that estimated using another one-year data, was used to measure the predictability (Table 4). Within the four-year data of the Predictability Study, there were six distinct two-year combinations. For each combination, there were two years that could be predicted. Therefore, there were 12 distinct prediction pairs of two-year *vs* one-year. Comparing the predictability of two-year *vs* one-year and one-year *vs* one-year, as measured by the mean ρ of all prediction pairs within trails, the improvement of the mean ρ using two-year multiple-location data were significant, but varied among three trials. The improvement of the ρ was 0.10 points with a rate of 23.8% in GXE15, was 0.06 points with a rate of 8.6% in GXE25, and was 0.09 points with a rate of 13.6% in GXE35.

For the predictability of three-year *vs* one-year within trials, the ρ between the BLUPs estimated using three-year multiple-location data and that estimated using another one-year data was used (Table 5). There were four distinct three-year combinations. For each combination, there was only one one-year multiple-location data that could be predicted. Therefore, there were four distinct prediction pairs of three-year *vs* one-year. Comparing the predictability of three-year *vs* one-year and two-year *vs* one-year as measured by the mean ρ of all predictability pairs within trials, the improvements of the mean ρ using three-year multiple-location data were very slight for three trials. The

improvement of the ρ was 0.02 points with a rate of 3.8% in GXE15, was 0.04 points with a rate of 5.3% in GXE25, and was 0.03 points with a rate of 4.0% in GXE35.

The results from three trials indicated that two-year multiple-location trials could significantly improve the predictability of the test-line performances in another year measured by the ρ compared to one-year multiple-location trials, if a trial had relatively high GxYxL variation contribution, and relatively low G variation contribution. If a trial had relatively low GxYxL variation contribution, and relatively high G variation contribution, an additional one-year multiple location trial might provide a very slight improvement of the ρ, one-year multiple-location trials might be sufficient to achieve a relatively high Spearman's ρ.

Spearman's ρ is a widely used statistics in research to measure the correlativeness between two variables along with Pearson's r. Any rank change associated with any element, no matter at top or bottom tier of the group, would equally influence the value of ρ. From a breeding perspective, breeders would only pay attention to the lines at top tier performance for a trait. In this study, the selections of the top 25% lines based on the BLUPs of the yield estimated using four-year multiple-location trials were considered as true genetic high-yielding lines. The selections also were done based on the BLUPs estimated using the data of one-year, two-year, and three-year multiple-location trials with the same selection intensity of 25%, separately. The number of the overlapping selection lines with these based on four-year multiple-location trials was used to measure the predictability of the trials under each of the scenarios. In GXE25, one-year multiple-location trials had sufficient power to capture the high-yielding lines with true super genetics, but, in GXE15 and GXE35, two-year multiple-location trials might be needed to

assure sufficient power to capture the high-yielding lines with true super genetics. The three-year trials did not provide any more merit to capture the high-yielding lines compared to the two-year trials (Table 6). The results indicated that, given a selection intensity, the one-year multiple-location trials might not be sufficient to capture the true genetic high-yielding lines if a trial had relatively high GxE effect, whereas, the two-year multiple-location trials would be sufficient to account for GxE influence in top-yielding line selections by capturing the effect of GxYxL. Based on the analysis of two-year balanced sub-set of soybean MYLT data in RM group V and VI from the North Carolina Official Variety Trials, Bowman (1998) drew the similar conclusion that two-year multiple-location trials would be appropriate to use in selecting the high-yielding lines. This result is coincident with the common practice in crop breeding that, to avoid the lose of the true high-yielding lines, breeders make selections using lower selection intensity to select more lines in the early stages of multiple-location trials comparing to late stages. And, the commercial nominations at late stage would be based on two-year multiple-location trials.

CONCLUSIONS

The study was conducted to dissect GxE effect into GxY, GxL, and GxYxL, and to determine whether there is any predominant source among them or not, and with the given results, to evaluate the predictability of soybean multiple-location trials with different number of years. The results indicated that i) the GxE is omnipresent with varied percentage of the explained variation contribution among the total observed yield variation, and the predominant source of GxE is GxYxL; ii) the significant presence of GxE and YxL warrants multiple-year and multiple-location trials for sufficient predictability of

the line performances in the following year. However, two-year multiple-location trials should be sufficient to capture the soybean top-yielding lines. If a trial with relatively low presence of GxE, one-year multiple-location trial should be sufficient to capture the soybean top-yielding lines.

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Table 1 Variance components of the observed yield from analysis of multiple location trial data by trial and year.

Trial	Variable	Variance (kg ha^{-1})^2	Percentage of total†	StdErr (kg ha^{-1})	Z-Value	P-value‡
		Year 2009				
GXE15	Location (L)	265858.1**	76.0	1505.17	2.63	0.004
	Line (G)	12918.7**	4.5	74.99	2.56	0.005
	GxL	10182.4**	10.7	60.78	2.49	0.006
	Residual	57040.2***	8.8	69.45	12.21	1.33E-34
GXE25	L	890895.0**	81.1	4321.66	3.07	0.001
	G	36821.4**	4.1	199.92	2.74	0.003
	GxL	30372.5***	8.8	119.06	3.79	7.43E-05
	Residual	116336.7***	6.1	125.58	13.78	1.78E-43
GXE35	L	252861.4**	57.2	1417.40	2.65	0.004
	G	27830.8*	7.4	181.84	2.28	0.011
	GxL	37692.6***	21.0	156.75	3.58	1.75E-04E
	Residual	122907.8***	14.4	157.44	11.61	1.87E-31
		Year 2010				
GXE15	L	225031.9**	69.7	1239.73	2.70	0.003
	G	3243.9^{ns}	2.2	35.94	1.34	0.090
	GxL	36133.3***	19.3	91.40	5.88	2.07E-09
	Residual	56316.5***	8.8	69.74	12.01	1.62E-33
GXE25	L	264438.1**	65.6	1259.99	3.12	0.001
	G	22252.4**	6.1	123.61	2.68	0.004
	GxL	22951.3***	16.5	91.16	3.74	9.06E-05
	Residual	93271.7***	11.8	97.42	14.24	2.70E-46
GXE35	L	377892.6**	73.4	2002.77	2.81	0.003
	G	27344.5**	6.1	149.41	2.72	0.003
	GxL	23282.5***	11.8	85.08	4.07	2.36E-05
	Residual	77986.4	8.7	85.31	13.59	2.17E-42
		Year 2011				
GXE15	L	354917.1**	77.0	1945.08	2.71	0.003
	G	8279.3*	2.6	61.53	2.00	0.023
	GxL	35074.0***	13.7	95.80	5.44	2.61E-08
	Residual	63721.0***	6.8	78.95	12.00	1.76E-33
GXE25	L	278135.4**	72.3	1321.03	3.13	0.001
	G	19139.9**	5.6	105.27	2.70	0.003
	GxL	24845.6***	13.9	69.59	5.31	5.52E-08
	Residual	61359.7***	8.2	64.15	14.22	3.33E-46
GXE35	L	280406.1**	71.3	1404.52	2.97	0.001
	G	17662.3**	5.3	100.25	2.62	0.004
	GxL	23977.0***	14.3	78.97	4.51	3.17E-06
	Residual	69378.1***	9.0	76.42	13.50	7.89E-42

Continued next page.

Table 1 Continued.

		Year 2013				
GXE15	L	555023.1**	84.9	3384.37	2.44	0.007
	G	10675.1*	2.2	70.18	2.26	0.012
	GxL	14710.0**	7.2	82.93	2.64	0.004
	Residual	69688.6***	5.7	91.16	11.37	3.05E-30
GXE25	L	492987.4**	75.2	3015.56	2.43	0.008
	G	42987.2**	7.2	236.41	2.70	0.003
	GxL	32012.3***	10.7	121.22	3.93	4.30E-05
	Residual	85445.6***	6.8	114.37	11.11	5.66E-29
GXE35	L	181208.1**	63.7	1070.05	2.52	0.006
	G	29442.5**	11.4	158.07	2.77	0.003
	GxL	18046.4***	15.0	69.53	3.86	5.68E-05
	Residual	53452.2***	10.0	67.91	11.70	6.02E-32

*, **, *** Z test of equal to zero significant at P = 0.05, P = 0.01, and P = 0.001, respectively; ns: non-significant at P = 0.05.

† Calculated from sum of squares estimated from general linear model with all factors as fixed effects.

‡ 1.33E-34 = 1.33 x 10^{-34}; all the other scientific notation in the same logic.

Table 2 Variance components of the yield from analysis of multiple year/location trial data; and partition of the variance of GXE.

Variable		Variance $(kg\ ha^{-1})^2$	Percentage of total†	StdErr $(kg\ ha^{-1})$	Z-Value	P-value‡
			GXE15			
Year (Y)		0.0				
Location (L)		107759.7*	37.0	930.20	1.72	0.042
Line (G)		4805.4*	1.5	33.81	2.11	0.017
YxL		229871.7***	38.2	734.22	4.66	1.62E-06
	GxY	3884.2**	1.2	20.59	2.80	0.003
GxE§	GxL	4426.5**	3.9	25.90	2.54	0.006
	GxYxL	19829.7***	8.1	43.99	6.70	1.02E-11
Residual		61635.2	7.3	38.54	23.78	2.80E-125
			GXE25			
Y		0.0				
L		41820.5^{ns}	28.1	792.64	0.78	0.216
G		22279.4**	3.9	122.37	2.71	0.003
YxL		416435.1***	46.3	1197.65	5.17	1.17E-07
	GxY	7233.1***	1.2	30.90	3.48	2.50E-04
GxE§	GxL	7982.1***	4.3	32.39	3.66	1.24E-04
	GxYxL	19137.3***	6.8	51.39	5.54	1.53E-08
Residual		88980.3	7.4	49.37	26.80	1.70E-158
			GXE35			
Y		0.0				
L		45453.4^{ns}	24.2	566.92	1.19	0.117
G		20075.7**	5.6	108.56	2.75	0.003
YxL		226619.1***	42.2	696.44	4.84	6.54E-07
	GxY	4989.8**	1.6	26.30	2.82	0.002
GxE§	GxL	2413.7^{ns}	5.2	28.11	1.28	0.101
	GxYxL	23214.1***	10.4	53.12	6.50	4.08E-11
Residual		79655.7	10.6	46.89	25.26	4.10E-141

*, **, *** Z test of equal to zero significant at P = 0.05, P = 0.01, and P = 0.001, respectively.

† Calculated from sum of squares estimated from general linear model with all factors as fixed effects.

‡ 1.62E-06 = 1.62×10^{-6}; all the other scientific notations are in the same logic.

§ Interaction effect between line and environment.

Table 3 Spearman rank correlation coefficients (ρ) between the BLUP (best linear unbiased prediction) values obtained from the analysis of linear mixed model by year.

Year vs. Year	ρ of GxE15	ρ of GxE25	ρ of GxE35
2009 *vs* 2010	0.50	0.75	0.76
2009 *vs* 2011	0.40	0.72	0.74
2009 *vs* 2013	0.72	0.71	0.48
2010 *vs* 2011	0.16	0.73	0.85
2010 *vs* 2013	0.44	0.70	0.60
2011 *vs* 2013	0.31	0.59	0.55
Mean	0.42	0.70	0.66

Table 4 Spearman rank correlation coefficients (ρ) between the BLUP (best linear unbiased prediction) values obtained from the analysis of linear mixed model by year and by combined two-year data.

Year vs. Year	ρ of GxE15	ρ of GxE25	ρ of GxE35
2009+2010 *vs* 2011	0.32	0.78	0.85
2009+2010 *vs* 2013	0.60	0.78	0.58
2009+2011 *vs* 2010	0.37	0.81	0.87
2009+2011 *vs* 2013	0.65	0.73	0.58
2009+2013 *vs* 2010	0.48	0.78	0.85
2009+2013 *vs* 2011	0.39	0.69	0.83
2010+2011 *vs* 2009	0.66	0.76	0.76
2010+2011 *vs* 2013	0.47	0.66	0.61
2010+2013 *vs* 2009	0.80	0.81	0.71
2010+2013 *vs* 2011	0.27	0.74	0.87
2011+2013 *vs* 2009	0.72	0.84	0.65
2011+2013 *vs* 2010	0.45	0.77	0.81
Mean	0.52	0.76	0.75

Table 5 Spearman rank correlation coefficients (ρ) between the BLUP (best linear unbiased prediction) values obtained from the analysis of linear mixed model by year and by combined three-year data.

Year vs. Year	ρ of GxE15	ρ of GxE25	ρ of GxE35
2009+2010+2011 *vs* 2013	0.62	0.79	0.60
2009+2010+2013 *vs* 2011	0.31	0.75	0.88
2009+2011+2013 *vs* 2010	0.47	0.81	0.88
2010+2011+2013 *vs* 2009	0.76	0.84	0.74
Mean	0.54	0.80	0.78

Table 6 Overlapping selections with a selection intensity of 25% between the selections based on the combined four-year trials, and the selections based on single year, combined two-year, and combined three-year, respectively.

Data set		GxE15†	GxE25‡	GxE35§
One-year	2009	4	4	4
	2010	2	5	4
	2011	2	5	3
	2013	3	4	3
	Mean	2.8	4.5	3.5
Two-year	2009+2010	4	5	5
	2009+2011	4	5	5
	2009+2013	4	4	4
	2010+2011	3	5	4
	2010+2013	4	5	4
	2011+2013	4	5	4
	Mean	3.8	4.8	4.3
Three-year	2009+2010+2011	4	5	5
	2009+2010+2013	4	4	5
	2009+2011+2013	4	5	4
	2010+2011+2013	3	5	5
	Mean	3.8	4.8	4.8

† The predictability Study at RM region of 1.0 - 1.9

‡ The predictability Study at RM region of 2.0 - 2.9

§ The predictability Study at RM region of 3.0 - 3.9

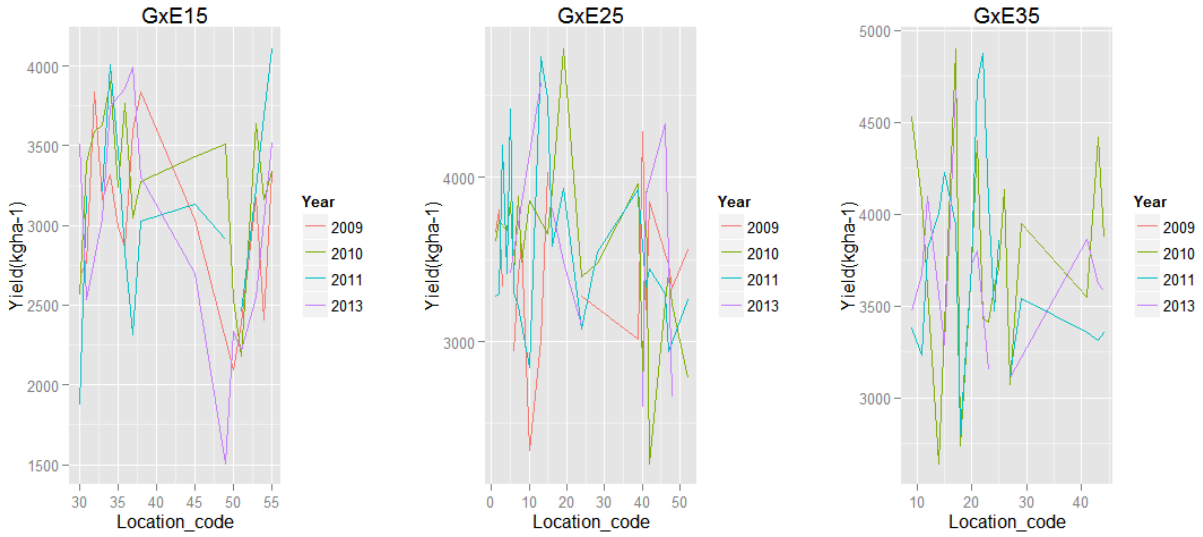

Fig. 1 Regressions between test locations and the mean yields of the locations measured by mean yields of testing lines within locations (location_codes were based on the alphabetical order of the first letter of test-location names across three trials).

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CHAPTER 4. THIN PLATE SPLINE SPATIAL MODEL USED AT EARLY STAGES OF SOYBEAN BREEDING TO CONTROL FIELD SPATIAL VARIATION: THE STUDY OF TEST EFFICIENCY IMPROVEMENT USING FIVE CHECKS WITHIN TEST-SETS

ABSTRACT

The early selection of superior plants with high-yielding potential is a major goal in soybean breeding programs. The progeny-row yield trial (PRYT) is usually conducted as a means to assess the yield potential of F_3-derived progenies. The yield variation observed in a PRYT is the final result of the line's genotypic merit, the field spatial pattern, and the experimental error. The spatial variation in field tests could confound the estimates of genetic merits, and might induce errors in selection of superior lines, thus decreasing the estimates of genetic gain per year. The objectives of this research were to: determine whether, by increasing the number of checks and with random arrangement within test-sets in the PRYT, the two-dimensional thin plate spline spatial model (TPS) could improve the selection efficiency on the basis of individual line performance. The second objective was to evaluate whether, the application of more checks and with random arrangement within test-sets could further increase the estimation accuracy in the PRYT on the basis of population mean performance. In the Spatial Study in 2013, 1161 lines were evaluated within test-sets along with five checks in progeny-row plots. The TPS model first was described by Bookstein in 1989. Our results indicated that the use of the TPS in the Spatial Study might prove to be effective in reducing the error variance based on the combined two-location data, with an improvement in relative efficiencies (IRE) of 56.7% and 65.9% in treatments 1 and 3, respectively, whereas, the IREs were 8.8% and 4.8% in treatments 2

and 4, respectively. The same group of 1161 lines also was tested in four-location tests with two-row plots, the Conformation Study. The correlation coefficients calculated between yield estimates, obtained in the Spatial Study with the TPS adjustment and in the Confirmation Study, did not improve compared to results from the non-TPS experiments. The results indicated that the use of the TPS spatial was not effective in increasing the selection efficiency in the Spatial Study on the basis of population mean performance and individual line performance, However, some improvement in reducing experimental error variance based on the analysis of variance with the combined two-location data was noticed. The factors to be considered in interpreting the results refer to the environmental conditions at the research site in Central IA from reproductive stage R3 to R6, and the growing conditions at the research site in Southeast NE for the Spatial Study. The field in Central IA, the extreme drought during the pod-development in 2013 damaged the field experiment, along with the summer drought in 2012, which resulted in a deficient of soil moisture in 2013 growing season. In test field in Southeast NE, the issues with field management were observed, especially, in test-field 2 where treatments 2 and 4 were allocated, on October 4, 2013 before harvest.

Keywords Progeny-row yield trial, genetic gain, two-dimensional thin plate spline, best linear unbiased prediction, soybean breeding

Abbreviations and Nomenclature (PRYT) progeny-row yield trial; (CV) coefficient of variation; (SRMSD) square root of mean square deviation; (SRMSE) square root of mean sum of squares for error; (TPS) two-dimensional thin plate spline; (IRE) improvement in

relative efficiency; (IQR) interval quartile range; (yld_adj) yield with the TPS spatial effect adjustment; (BLUP) Best linear unbiased prediction; (RM) relative maturity.

In this publication, data from Monsanto Inc. have been used with permission.

INTRODUCTION

In plant breeding, for a target trait, the genetic gain per year is the primary focus to measure the success of a breeding program (Fehr, 1993). To achieve the high genetic gain for low heritability traits, such as yield, plant breeders in self-pollinated crops have modified the breeding methods to allow yield selection as early as possible in the early generation of segregating populations (Bell, 1963; Shebeski, 1967; Boerma and Cooper, 1975). The progeny-row yield trail (PRYT) is commonly used in early generation tests. The efficient of the PRYT in self-pollinating species as a means to identify superior genotypes has been reported in different crops with varying success. Based on the research from chickpea (*Cicer arietinum* L.), Dahiya et al. (1984b) concluded that an early generation yield-testing selection procedure based on one-location and single progeny-row plot tests was effective. DePauw and Shebeski (1973) assessed the yield correlation and regression coefficients between wheat (*Triticum aestivum* L.) yields estimated based on F_3 progeny-row yield tests and the related bulk means of F_4 and F_5 lines from multiple location tests. They concluded that the progeny-row yield trials could discriminate among F_3 lines for heritable quantitative differences. However, in the research with cowpea (*Vigna unguiculata* (L.)), Padi and Ehlers (2008) did not find a correlation between yield based on unreplicated F_3 individual plant data tested at one-location and their yield

performance in F_4 bulks tested at three locations. They concluded that early generation selection for yield was ineffective in cowpea based on single-plant plot yield tests.

In soybean breeding, however, reports from early generation studies, while generally favorable, gave mixed results. Cooper (1990) reported that, based on the reduced number of F_2-derived lines per cross, and the use of single location, single replication data for the selection in $F_{2,3}$ through $F_{2,3,4}$, eight high-yielding soybean cultivars were released. Streit et al. (2001) reported that the soybean seed yield selection based on the PRYT was as effective as based on replicated multiple environment tests for all four populations used in their research. Hegstad et al. (1999) concluded that PRYT test would make the progress in identifying elite soybean lines with true high potential. However, the correlations of yield in the PRYT and replicated tests in only two out of five of their populations were significantly positive.

The varying levels of effectiveness reported in different studies might be the result of the differences in selection procedures and criteria, population heterozygosity, heritability of traits, and statistical analyses. Bernardo (2003) conducted a simulated study for self-pollinated crops and concluded that early generation tests and PRYT-based selection of lines were expected to be effective in predicting performance of genotypes, unless non-genetic effects were large relative to the true genetic merits of the tested lines. Bernardo's observations suggest that test size might be an important factor that could affect the prediction value of the early generation tests. According to Bernardo (2003), the observed yield variation in the PRYT resulted from the genotypes of soybean lines, and the addition of variable non-genetic effects, such as field spatial patterns, and experimental error. A large yield variation in soybean PRYT, due to non-genetic effects, was observed

(Sun, 2014). The variation due to non-genetic effects could be a confounding factor in determining the genetic variation in yield of the test lines (Becker, 1995).

Sun (2014) implemented the TPS spatial modeling in a designed PRYT study and in a large scale of soybean PRYT. The use of the TPS was effective in reducing the error variance and the coefficient of variability, with an improvement in relative efficiency (IRE) of 37.9% in a designed PRYT study. In the large scale of soybean PRYT, the TPS model also was effective with the IRE of 40.4%. The correlation coefficients, calculated between yield estimates obtained in the two-year PRYTs and the four-location tests with two-row large plots, improved by 0.21 units on the basis of population mean performance due to the TPS spatial adjustment compared to results from the non-TPS experiments. These results indicated that the use of the TPS spatial was effective in reducing the spatial variation in field tests. However, limited by the number of checks used in the research, the adjustments obtained by the TPS were not effective in increasing the selection efficiency of the PRYT on the basis of individual line performance. Research was designed to determine if, or not, by increasing the number of checks and with random arrangement within test-sets in the PRYT, the two-dimensional thin plate spline spatial model (TPS) could improve the selection efficiency on the basis of individual line performance. The second objective was to evaluate if, or not, the application of more checks and with random arrangement within test-sets could further increase the estimation accuracy in the PRYT on the basis of population mean performance.

MATERIALS AND METHODS

The research consisted of two separate experiments of the Spatial Study and the Confirmation Study. The Spatial Study was designed to assess the efficiency of the TPS

spatial model under different number of checks used and the different arrangement of the checks within test-sets as measured by i) the improvement in relative efficiency (IRE) between the actual yield test and the adjusted yield test through the TPS modeling; ii), the efficient of a yield correction factor for the spatial effect adjustment. The Confirmation Study was designed to assess the efficiency of the TPS spatial model by comparing the performance of bi-parental populations and individual lines evaluated in the Spatial Study and their later evaluation in multiple location tests. The line and population performance estimates obtained from the Confirmation Study were considered to provide better estimates of the true genetic performance of the lines, since these estimates were derived from replicated multi-location trials.

In the conduct of the two experiments, field plots were planted and harvested mechanically, utilizing computer technology to automatically record grain harvest and grain moisture. The agronomic data recorded in each individual progeny-row plot were relative maturity (RM) at reproductive stage R7 (Fehr et al., 1971), and grain yield measured in kg ha^{-1} at reproductive stage R8 (Fehr et al., 1971) with a moisture correction factor to express yield at 13.5% moisture content. The individual plots in each experiment were all harvested in bulk.

Spatial Study

The Spatial Study was conducted in 2013 at two Monsanto Inc. soybean research stations located in Central IA and Southeast NE. The soil types were sandy clay loam at research location in Central IA and Silt Loam at research location in Southeast NE. The sowing dates were June 7 at location in Central IA and June 12 at location in Southeast NE.

The climate information of the two locations is presented at Table 1. Nine bi-parental populations each represented by 129 F_3-derived lines in $F_{3:5}$ were evaluated, for a total of 1161 $F_{3:5}$ lines. The 129 lines per population were randomly divided into three groups of 43 lines each, to which five checks were added, bringing the total to 48 entries per test-set. Each of the nine bi-parental populations was represented by three test-sets in three replicates. Three of the five checks formed three check-sets with three replicates as well. Each check-set contained one check replicated 44 times, and four other checks replicated only once. The nine test-sets and the three check-sets were assigned into three blocks. Within a block, the test-sets and the check-sets were arranged randomly. The three blocks formed one test-field within a test location. In the same test location, the same test-sets and check-sets with the same group of test lines and checks formed another test-field. Two test-fields were adjacent in a location. The arrangement of the five checks within test-sets of a test-field was random, and was different from the arrangement of the checks at another test-field. But, the random patterns of the checks within a test-field were consistent across the test-sets and check-sets. The same random pattern of the check arrangements within a test-field formed a treatment. The two test-fields with two different patterns within one location formed two treatments of treatment_1 and treatment_2.

In treatment_1, the arrangement of five checks within test-sets or check-sets was: check_1 was placed at ranges 1 of column 1, check_2 at range 1 of column 2, check_3 at range 2 of column 1, check_4 at range 4 of column 12, and check_5 at range 3 of column 8. In treatment_2, the arrangement of five checks was: check_1 was placed at ranges 2 of column 6, check_2 at range 4 of column 12, check_3 at range 1 of column 1, check_4 at range 1 of column 11, and check_5 at range 3 of column 2. Upon harvest, treatment_3 and

treatment_4 were simulated by setting yield values of check_4 and check_5 as missing values from treatment_1 and treatment_2, respectively.

Across four treatments, within test-sets and check-sets, the 48 entries were arranged in progeny-row plots in a grid four ranges deep and 12 columns wide. Row-plots were planted at 40 seeds per plot in 1.5 m row length, spaced 0.8 m between rows resulting in a sowing population of 27 seeds m^{-1}.

Confirmation Study

Each of the 129 $F_{3:5}$ lines per bi-parental population (nine populations) along with other 1404 $F_{3:5}$ lines from other 10 bi-parental populations were planted in the Confirmation Study in 2011. The study was conducted at four locations (Central IA, Northwest IA, Eastern IA, and Southwest IA) at the Monsanto Inc. research sites. The soils at four locations were sandy clay loam, clay loam, silt clay loam, and clay loam, respectively. The sowing dates were May 10, May 9, May 7, and May 10, respectively. The 2565 lines were classified into three maturity groups, defined on the basis of RM recorded in 2010. The group with RM from 2.0 to 2.4 was composed of 405 lines and identified as F24S. The second RM group was from RM 2.5 to 2.8, composed of 1215 lines, and identified as F26S. The third group was identified as F29S and had RM from 2.9 to 3.1, and was composed of 945 lines. Within each RM group, 45 F_3-derived lines in $F_{3:6}$ were assigned randomly to one test-set, and five checks were added, bringing the total to 50 entries per test-set. Within a test-set, the 50 entries were planted randomly in the field in a grid five ranges deep and 10 columns wide. Plots were two-row plots, 3.7 m in length, and 0.8 m between rows resulting in a sowing population of 30 seeds m^{-1}.

Statistical Analysis

The assumptions of normality and homogeneity of variance in the error term of yield data from each of the two experiments were evaluated before any parametric statistical analysis was conducted. In all experiments, the yield data were continuous with near normal distribution based on the test of QQPLOT in the PROC UNIVARIATE procedure of SAS version 9.3.2 (SAS Institute, Cary, NC).

Box-plots based on the interval quartile range (IQR) method (Tukey, 1977) were implemented for outlier identification. IQR was calculated on a whole experiment base for the Spatial Study. IQR for the Confirmation Study was calculated for each individual location. On the basis of the research conducted by Tukey (1977) and Eo et al., (2012), an observed yield value is treated as an outlier when

$$y < q1 - 1.5*iqr \text{ or } y > q3 + 1.5*iqr \quad [1]$$

where y is the observed yield; q1 was the 25% sample quartile, q3 was the 75% sample quartile, and IQR was the difference between q3 and q1, for a given data set. An outlier is, therefore, treated as a missing value, otherwise, to avoid distorting the statistical inference. The yield data from each of the three experiments was analyzed using the SAS 9.2.3 statistical package (SAS Institute, Cary, NC), and R FIELDS package (Nychka, 2013).

In the Spatial Study, the yield variation observed within a check-set was assumed to consist of the field spatial patterns and the experimental error. The variation observed within a test-set was assumed to consist of the field spatial patterns, the experimental error, and the genetic variation among the test lines. The yield data set, containing only the checks, first was fitted into the general linear model with line as a fixed effect

$$y_{ijk} = \mu + v_k + e_{ij} \quad [2]$$

where y_{ijk} is the observed yield of the check k at range i and column j; μ is the overall mean; v_k is the check k effect; and e_{ij} is a random error having a normal distribution $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. Assuming the estimated residual of each plot, from this fitted model, as a combination of spatial patterns and experimental error, the TPS was implemented with the residuals to separate the spatial patterns from the experimental error.

The TPS model is a semi-parametric spatial model (Bookstein, 1989). Under the TPS model, the spatial trend effect at each progeny-row plot can be estimated as a function of its neighboring check plots, referred to as knots, by using a localized interpolation function (Robbins et al., 2005). The resulting TPS model corresponded to a mixed linear model for yield of line i in range j and column k given as

$$y_{kijl} = \mu + \sum_{l=1}^{n} W_{ijl}\beta_l + v_k + r_i + c_j + e_{kijl} \quad [3]$$

where μ is the overall mean; β_l is a fixed effect for the l-th knot; W_{ijl} is the weight for the l-th knot at range i and column j; v_k is the genetic effect for the k-th line; r_i is the random effect for the i-th range; c_j is the random effect for the j-th column; and e_{kijl} is a random error for the plot at range i and column j with $e_{kijl} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2 (Robbins et al., 2012).

The weight W_{ijl} is defined as

$$W_{ijl} = \frac{1}{\left\| R_l - R_i, C_l - C_j \right\|}, \quad [4]$$

where R_l and C_l are the range and column for the l-th knot; respectively; and R_i and C_j are the range and column for the plot on which the spatial effect β_l will be estimated,

respectively, the expression $\|a, b\| = \sqrt{a^2 + b^2}$ is Euclidian distance. The spatial effects were estimated using the R package FIELDS of version 6.9.1 (Nychka, 2013).

The spatial patterns obtained from the Spatial Study were used as a correction factor to adjust the observed yield on the basis of progeny-row in the Spatial Study. The observed yield after adjustment of the spatial effects predicted from the TPS was denoted as yld_adj.

In the Spatial Study, within each treatment across two locations, the analysis of variance with the following model was conducted using RM as a covariate:

$$y_{ij} = \mu + RM + v_i + l_j + vl_{ij} + e_{ij} \quad [5]$$

where y_{ij} is the yield or the yld_adj of line i in location j; μ is the overall mean; v_i is the line i effect; l_j is the location j effect; vl_{ij} is the line and location interaction effect; and e_{ij} is a random error having a normal distribution $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. Data were analyzed using the PROC GLM procedure of SAS version 9.3.2 (SAS Institute, Cary, NC). Yield or yld_adj were analyzed using the same model as described in equation [5] with the linear mixed model procedure (PROC Mixed) of SAS version 9.3.2 (SAS Institute, Cary, NC) where v_i and l_j were the random effects, and RM was used as a covariate. The best linear unbiased prediction (BLUP) of each line in each of the nine bi-parental populations was predicted based on the combined two-location data within each of the four treatments. The performance of the population was the mean performance of the lines within the population.

For individual location analysis of the Spatial Study, the following model was assumed with RM as a covariate and the effects of line and block as random effects:

$$y_{ij} = \mu + RM + v_i + b_j + e_{ij} \quad [6]$$

where y_{ij} is the yield or yld_adj of line i at block j; μ is the overall mean; v_i is the line i effect; b_j is the block j effect; and e_{ij} is a random error having a normal distribution $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. The BLUP of each line was estimated by location, and the performance of the population was estimated by location using the mean performance of the lines within populations.

In the Confirmation Study, yield data were analyzed across locations using the linear mixed model with RM as a covariate and the effects of lines and locations as random effects:

$$y_{ij} = \mu + RM + v_i + l_j + e_{ij} \quad [7]$$

Where y_{ij} is the yield of line i in location j; μ is the overall mean; v_i is the line i effect; l_j is the location j effect; and e_{ij} is a random error having a normal distribution $e_{ij} \sim$ independent $N(0, \sigma_e^2)$ with mean = 0 and variance = σ_e^2. Yield was analyzed using the PROC Mixed procedure of SAS version 9.3.2 (SAS Institute, Cary, NC). The BLUP was estimated for each line. The performance of the population was the mean performance of all the lines tested within bi-parental populations.

To assess the efficiency of using the TPS model in correcting yield in the Spatial Study, the IRE was calculated as:

$$IRE_{TPS} = \frac{(SRMSE_{non-TPS} - SRMSE_{TPS})}{SRMSE_{non-TPS}} \times 100\%, \quad [8]$$

where $SRMSE_{non-TPS}$ is SRMSE from the model without the TPS spatial adjustment, and $SRMSE_{TPS}$ is SRMSE from the model with the TPS spatial adjustment, multiplied by 100%, to express the value as percentage.

The Pearson correlation coefficients calculated between the performance in the Spatial Study, by location and combined-location within treatments, and that in the Confirmation Study also were used to measure the efficiency of the TPS model on the basis of individual progeny-row and bi-parental population. For the individual progeny-row performance, across nine bi-parental populations, based on the Spatial Study by location and combined-location, within each treatment, the top 5% lines were selected based on the yield without TPS adjustment and with TPS adjustment. The performance the selected lines were confirmed by the Confirmation Study by the overlapping number of the lines.

RESULTS AND DISCUSSION

Spatial Study

Quantification of Spatial Patterns Using Checks in the Spatial Studies. In the fields in Central IA and Southeast NE, the field spatial patterns were evaluated using a sub-set of data only containing the common checks. The analysis of residuals from the linear model with checks as fixed effects revealed that the yield distribution of the checks was affected by their spatial location within the field.

In test-field 1 of the Spatial Study in Central IA where treatments 1 and 3 were allocated, the residuals for the checks varied across field, the magnitude of the variation captured by treatment 1 was larger than treatment 3 (Fig. 1 and Fig. 3). In test-field 2 where the treatments 2 and 4 were allocated, based on both treatments, the residuals for the checks placed in the middle of the field were much smaller compared to the residual of the checks in the outer circle of the test-field (Fig. 2 and Fig. 4).

In test-field 1 of the Spatial Study in Southeast NE where treatments 1 and 3 were allocated, based on both treatments, the residuals for the checks placed in the middle of the field from upper side to the bottom side were larger than the ones placed in two sides of the field (Fig. 5 and Fig. 7). In test-field 2, where treatments 2 and 4 were allocated, the residuals for the checks placed in the upper area of the field were smaller compared to the residual of the checks in the lower area of the field (Fig. 6 and Fig. 8).

To quantify the field spatial patterns in test-yield separately by treatment in two test locations in Central IA and Southeast NE, the spatial effects across the test fields were predicted based on the TPS model with check residuals (Fig. 1 to Fig. 8).

In Central IA, in test-field 1, the spatial patterns predicted from treatments 1 and 3 were different (Fig. 1 and Fig. 3). Treatment 1 captured the spatial patterns with more detail compared to treatment 3. The predicted spatial effects ranged from -1253.0 kg ha^{-1} to 1248.9 kg ha^{-1}, with a SRMSD of 443.2 kg ha^{-1} for treatment 1. For treatment 3, the predicted spatial effects ranged from -897.9 kg ha^{-1} to 1201.5 kg ha^{-1}, with a SRMSD of 366.8 kg ha^{-1}. In test-field 2, the spatial patterns predicted from treatments 2 and 4 were similar (Fig. 1 and Fig. 3). The predicted spatial effects ranged from -1238.6 kg ha^{-1} to 1242.2 kg ha^{-1}, with a SRMSD of 541.9 kg ha^{-1} for treatment 2. For treatment 4, the spatial effects ranged from -1296.7 kg ha^{-1} to 1288.4 kg ha^{-1}, with a SRMSD of 560.8 kg ha^{-1}.

In Southeast NE, in test-field 1, the spatial patterns predicted from treatments 1 and 3 were similar (Fig. 5 and Fig. 7). Treatment 1 captured the spatial patterns with more detail compared to treatment 3. The spatial effects ranged from -1163.3 kg ha^{-1} to 596.8 kg ha^{-1}, with a SRMSD of 425.1 kg ha^{-1} for treatment 1. For treatment 3, the spatial effects ranged from -1191.0 kg ha^{-1} to 732.1 kg ha^{-1}, with a SRMSD of 494.8 kg ha^{-1}. In test-field 2,

the spatial patterns predicted from treatments 2 and 4 were much different with the magnitude of the spatial effect variation (Fig. 6 and Fig. 8). The spatial effects ranged from -740.8 kg ha^{-1} to 608.2 kg ha^{-1}, with a SRMSD of 299.4 kg ha^{-1} for treatment 2. For treatment 4, the spatial effects ranged from -594.4 kg ha^{-1} to 943.4 kg ha^{-1}, with a SRMSD of 318.0 kg ha^{-1}.

TPS Efficiency in Determining Spatial Effects. Across two locations and by treatment, the data with and without spatial adjustment (yld_adj and yield) were fitted into the general linear model for the analysis of variance using the model in equation[5]. For each treatment, the mean square for error from the analysis of yld_adj was smaller than that of the yield (Table 2), and the IRE_{TPS} was 56.7%, 8.8%, 65.9%, and 4.8% for treatment 1, treatment 2, treatment 3, and treatment 4, respectively. Based on the IRE_{TPS}, treatments 1 and 3 could capture spatial variation much better when compared to treatments 2 and 4, whereas, treatments 2 and 4 did not show significant improvement. This indicated that the random arrangement of the checks within test-sets did not capture the spatial variation better, and it was even less effective compared to non-randomization. There were no significant differences between treatments 1 and 3, and between treatments 2 and 4. This indicated that more checks arranged within test-sets did not provide any merit for capturing spatial variation. The results of the Spatial Study indicated that the effectiveness of the TPS spatial model varied among the treatments.

Confirmation Study

Selection Efficiency Improvement Using the TPS Spatial Model. In a soybean breeding program, the rows of the un-replicated PYRT test would be bulk-harvested and

the superior high-yielding genotypes would be selected for planting larger plots at multiple locations in the following year. In this study, all genotypes tested in the Spatial Study were planted in 2011 at each of four locations in Iowa in replicated field trials, with two-row plots for every genotype. All lines from the Spatial Study were planted in the Confirmation study to be able to compare the individual line yield performance in each of the tests planted.

The first yield performance comparison was made within each treatment by calculating the Pearson correlation coefficients between the mean performance of the individual bi-parental populations, in the individual locations of the Spatial Study, for the observed yield and the yld_adj, and that of the same populations evaluated in the Confirmation Study (Table 3). In the data-sets of the Spatial Study in Central IA and Southeast NE, among four treatments, the correlation coefficient of the estimated yield had no correlation coefficient that was significantly large than zero with the four-location Confirmation Study regardless whether the TPS adjustment was used or not. Across treatments and locations, with the TPS adjustment, the correlation coefficients ranged from -0.25 to 0.24 with a mean of -0.09, and a STD of 0.20. Without the TPS adjustment, the correlation coefficients ranged from -0.20 to 0.45 with a mean of 0.09, and a STD of 0.26. The difference of the mean correlation coefficients between TPS adjustment and no TPS adjustment was -0.18 ($P = 0.076$, for the difference of zero).

On the basis of individual line performance, within each treatment of each test location, the yield comparison was made by calculating the Pearson correlation coefficients between the Spatial Study for the observed yield and the yld_adj, and the Confirmation Study for the observed yield and the yld_adj of the same lines evaluated (Table 3). In the

data-sets from Central IA and Southeast NE, among four treatments, the correlation coefficients were significantly larger than zero regardless whether the TPS adjustment was used or not. Across treatments and locations, with the TPS adjustment, the correlation coefficients on the basis of individual line performance ranged from 0.09 to 0.22 with a mean of 0.13, and a STD of 0.046. Without the TPS adjustment, the correlation coefficients ranged from 0.11 to 0.19 with a mean of 0.15, and a STD of 0.034. The difference of the mean correlation coefficients between TPS adjustment and no TPS adjustment was -0.02 (P = 0.311, for the difference of zero).

To assess if the TPS model adjustment used in the Spatial Study would have an effect on identifying individual line performance of yield, a 5% selection intensity was applied to the lines tested in the Spatial Study, both on the basis of actual yield and yld_adj within each treatment. The selected lines (50 lines) were ranked according to yield as evaluated in the Spatial Study from 1 to 50 (data not shown). The ranking of the selected lines then was compared to the rank of the same lines evaluated in the Confirmation Study. The selection was practiced across nine bi-parental populations and by treatment, within location and also across two locations. The rank correlations were calculated for each of the selection environments and the yield selection criteria, actual yield, and yld_adj. None of the rank correlations among the studies were significant; this indicated that the line ranking at each test was different. When the selections were done under each of the selection environments and the yield selection criteria using the data of the Spatial Study, only four to nine of the selected lines were among the superior 50 genotypes in the Confirmation Study regardless whether the TPS adjustment was used or not (Table 4).

The effectiveness of the TPS spatial adjustment, on both the performances of the population mean and the individual line, were not observed, although the TPS spatial adjustment might be effective in controlling spatial variation in reducing test error variance in treatments 1 and 3.

CONCLUSIONS

This research was conducted to determine if or not more checks along with random arrangement of the checks within test-sets could further improve the TPS efficiency in removing spatial effect in the early generation test, measured by the improvement of the superior line selection accuracy, which was not observed in the previous studies. The underlying assumption was that more checks and the random arrangement of the checks would capture the spatial variation better on the basis of progeny-row plots. The results of this research indicated that the TPS model might be an effective tool to improve experimental accuracy measured by the reduction of the test error variance. But, the TPS spatial modeling did not show the improvement of the selection accuracy on the basis of population mean and individual line performances. The correlation coefficient between the population performances estimated from the Spatial Study with the TPS adjustment and the performances estimated from the Confirmation Study was not improved compared to that without the TPS spatial adjustment. More checks with random arrangement did not provide any merit for improving the TPS efficiency. When a selection intensity of 5% was used to identify the highest yielding lines in the Spatial Study, and their performance in the Confirmation Study was determined, the number of the overlapping superior lines in both studies did not increase with the TPS adjustment, and even with more checks along with random arrangement of the checks used. The results indicated that under the conditions of

this research, there was no improvement in the selection efficiency after using the TPS spatial adjustment.

The factors to be considered in interpreting the results of the Spatial Study refer to the environmental conditions at the research site in Central IA from reproductive stage R3 to R6, and the growing conditions at the research site in Southeast NE.

The field in Central IA, the extreme drought during the pod-development in 2013 damaged the field experiment, along with the summer drought in 2012, which resulted in a deficient of soil moisture in 2013 growing season (Table 1). There was only 50.5 mm rain fall in July and August, whereas, the normal rainfall is more than 240.0 mm measured by the mean rain fall of the last 17 years for July and August. The growing season of 2012 from May 1 to September 30, in Central IA, a drought was observed with the total rain fall of 307.9 mm, whereas, normal rainfall in total is 566.5 mm, this caused the water supply deficient in the deep layer soil of the field. Two-year cumulative drought in Central IA, especially, the extreme drought during the pod-development in 2013, destroyed the field experiment in Central IA. The field photos taken on September 9 showed the damage of the plants (Fig. 9 and 10). In test-field 1, the pattern of yellow plants was observed like an inverse T (Fig. 9). During RM field record on October 12, the plots that fell into the T pattern and nearby the T pattern were partially dead or totally dead. In test-field 2, the plants at the middle of the test-field turned into yellow on September 9 (Fig. 10), and were observed dead on October 12. The damage of the experiment was investigated by Dr. Kevin Matson, Dr. Christiana Wiebbecke, and Minghui Sun (Monsanto Inc.) on October 9, we concluded that the dead plants were caused by the extreme water deficient. It was not caused by the diseases, nor the susceptibility of the plant genetics to the drought. For the

last four years since 2010 when Monsanto Inc. conducted field experiments in this site, a similar situation was not observed (Christiana Wiebbecke, personal communication, Monsanto Inc. 2013).

In 2013, in research site in Southeast NE, irrigation resolved the drought issue when there was shortage of rain fall (Table 1). On October 4 before harvest, Dr. Reid Palmer (Iowa State University) and Minghui Sun (Monsanto Inc.) visited the test field. The issues with field management were observed, especially, in test-field 2 where treatments 2 and 4 were allocated. This might explain the reason that the experimental accuracy, estimated based on treatments 2 and 4, and measured by the reduction of the experimental error variance, was not improved as much as that based on treatments 1 and 3 from the analysis of variance based on the combined two-location data.

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Table 1 Weather information for research locations of Monsanto Inc. in Central IA and Southeast NE that was used in the conduct of the Spatial Study in 2012 and 2013.

Location	Weather	Year	April	May	June	July	August	Sept.
Central IA	Rainfall (mm)	2012	107.2	57.5	71.9	56.6	67.3	54.6
		2013	152.5	247.8	99.8	33.2	17.3	50.1
		1997 to 2013†	101.8	143.7	131.2	103.7	110.0	77.9
	Temperature‡ (°C)	2012	11.5	19.2	22.4	26.7	21.8	17.1
		2013	6.7	15.1	20.9	22.6	22.6	19.8
		1997 to 2013†	9.9	16.0	21.1	23.7	22.3	17.8
Southeast NE	Rainfall (mm)	2012	79.2	80.8	48.5	10.8	27.7	19.0
		2013	65.4	137.2	55.1	32.9	47.1	47.3
		1997 to 2013†	74.0	114.7	105.5	75.8	73.7	49.9
	Temperature‡ (°C)	2012	13.3	19.1	23.5	27.2	22.6	18.1
		2013	7.0	15.6	21.6	23.3	23.4	20.8
		1997 to 2013†	10.5	16.6	21.8	24.8	23.4	18.6

† Averages of rainfalls and daily temperatures from 1997 to 2013.

‡ Daily temperature was the mean of daily maximum and minimum temperatures.

Note: The soil type of the research location in Central IA is sandy clay loam, the sowing date was June 7; The soil in the research location in Southeast NE is silt loam, the sowing date was June 12.

Table 2 Analysis of variance of the Spatial Study by treatment combined over two locations with relative maturity (RM) as a covariate based on yield observations without TPS spatial adjustment (yield) and with TPS spatial adjustment (yld_adj).

Source	DF	Mean square (kg ha^{-1})			
		Yield			
		Treatment 1[§]	Treatment 2[§]	Treatment 3[§]	Treatment 4[§]
RM	1	17154.0^{ns}	13216741.0***	411306.0^{ns}	6474881.0***
Line (V)	1473	852249.0***	892240.0***	823030.0***	868676.0***
Location (L)	1	311123113-0***	25698979***	208684144.0***	25493437.0***
V x L	1473	456703.0^{ns}	619839.0^{ns}	454989.0^{ns}	604058.0^{ns}
Error	1315	571838.4	582779.6	634093.7	565804.8
		Yld_adj			
		Treatment 1	Treatment 2	Treatment 3	Treatment 4
RM	1	218931.0^{ns}	4079283.0*	36206.0^{ns}	2222247.0*
Line (V)	1473	1298074982.0***	1006092.0***	908236.0***	977179.0***
Location (L)	1	378236730.0***	27070865.0***	393748831.0***	27770972.0***
V x L	1473	528771183.0***	631063.0**	432978.0***	664927.0***
Error	1315	247804.8	531441.0	215946.1	538902.8
IRE_{TPS}[†]		56.7%	8.8%	65.9%	4.8%

*, **, *** F test significant at P = 0.05, P = 0.01, and P = 0.001, respectively; ns F test non-significant at P = 0.05.

† $IRE_{TPS} = ((SRMSEyield - SRMSE_{yld\_adj}))/SRMSE_{yield} \times 100\%$.

§ Treatments were based on the number and arrangement of checks within test-set across test-field.

Table 3 Analysis on the basis of individual line performance and bi-parental population mean performance for Pearson correlation coefficients between the Confirmation Study, and the Spatial Study in each individual location with yield observations without TPS spatial adjustment (yield) and with TPS spatial adjustment (yld_adj), respectively.

Test	Individual line base		Population mean base	
	TPS adjustment (yld_adj)	NON-TPS adjustment (yield)	TPS adjustment (yld_adj)	NON-TPS adjustment (yield)
		Central IA		
Treatment 1	0.17***	0.19***	0.24^{ns}	0.09^{ns}
Treatment 2	0.09**	0.18***	-0.27^{ns}	0.45^{ns}
Treatment 3	0.22***	0.19***	0.20^{ns}	0.10^{ns}
Treatment 4	0.09**	0.18***	-0.25^{ns}	0.45^{ns}
		Southeast NE		
Treatment 1	0.14***	0.11***	-0.19^{ns}	0.13^{ns}
Treatment 2	0.11**	0.14***	-0.13^{ns}	-0.20^{ns}
Treatment 3	0.15***	0.11***	-0.08^{ns}	-0.12^{ns}
Treatment 4	0.10**	0.14***	-0.23^{ns}	-0.20^{ns}

*, **, *** F test significant at P = 0.05, P = 0.01, and P = 0.001, respectively; ns F test non-significant at P = 0.05.

Table 4 Analysis of performance on the basis of the top 5% selections (i.e. 50 lines) across populations based on the Spatial Study with and without TPS spatial adjustment for yield observations, by location and combined-location, for the number of lines overlapped in top 5% in the Confirmation Study.

Treatment	No._lines _TPS†	No._lines _NON‡	No._lines _TPS†	No._lines _NON‡	No._lines _TPS†	No._lines _NON‡
	Central IA		Southeast NE		Combine-locations	
Treatment 1	4	9	6	6	5	8
Treatment 2	5	6	7	5	7	4
Treatment 3	9	9	5	6	7	8
Treatment 4	5	6	7	5	3	4

† With the TPS spatial adjustment, the number of the lines that the top 5% selections based on Spatial Study overlapped with the top 5% in the Confirmation Study.

‡ Without the TPS spatial adjustment, the number of the lines that the top 5% selections based on Spatial Study overlapped with the top 5% in the Confirmation Study.

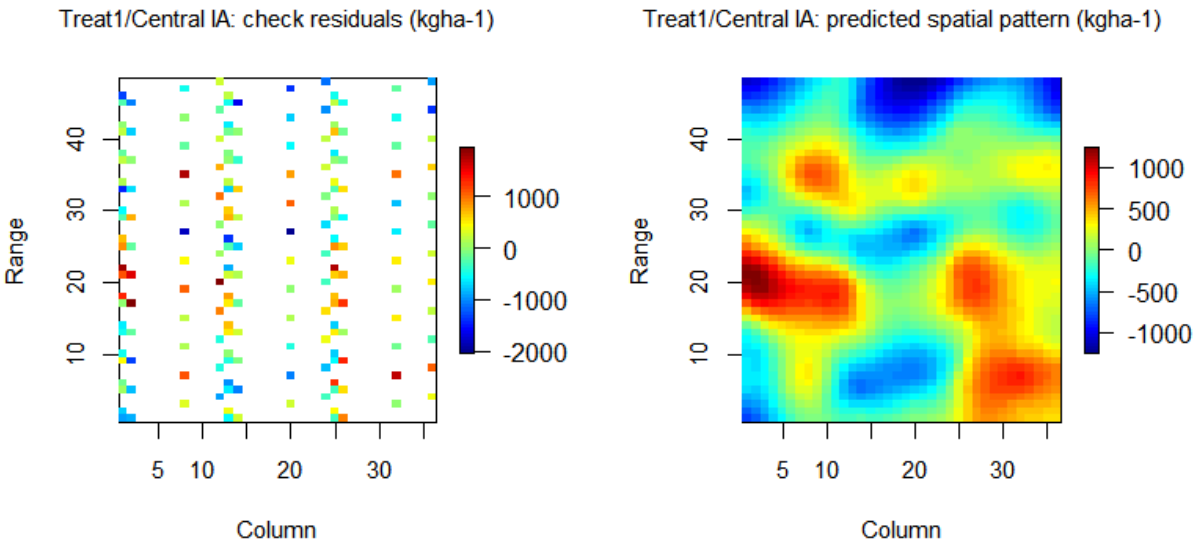

Fig. 1 Treatment 1 in Central IA. The heat map of check residuals estimated from the linear model with checks as fixed effects based on the yield of checks of the Spatial Study in 2013 (left), and the heat map of the predicted spatial pattern using the TPS spatial model based on the check residuals from the Spatial Study (right).

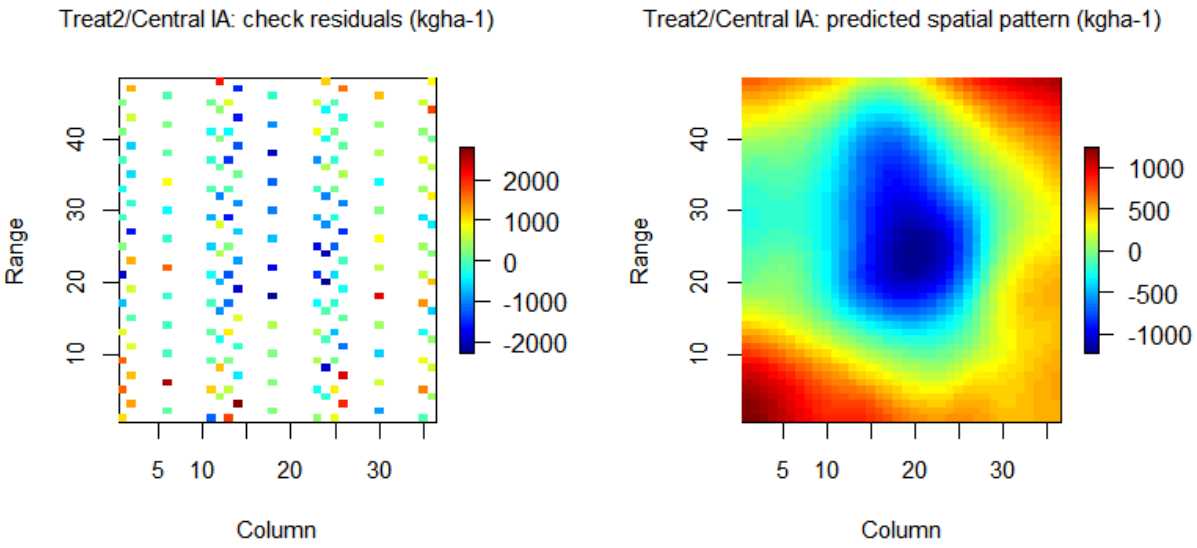

Fig. 2 Treatment 2 in Central IA. The heat map of check residuals estimated from the linear model with checks as fixed effects based on the yield of checks of the Spatial Study in 2013 (left), and the heat map of the predicted spatial pattern using the TPS spatial model based on the check residuals from the Spatial Study (right).

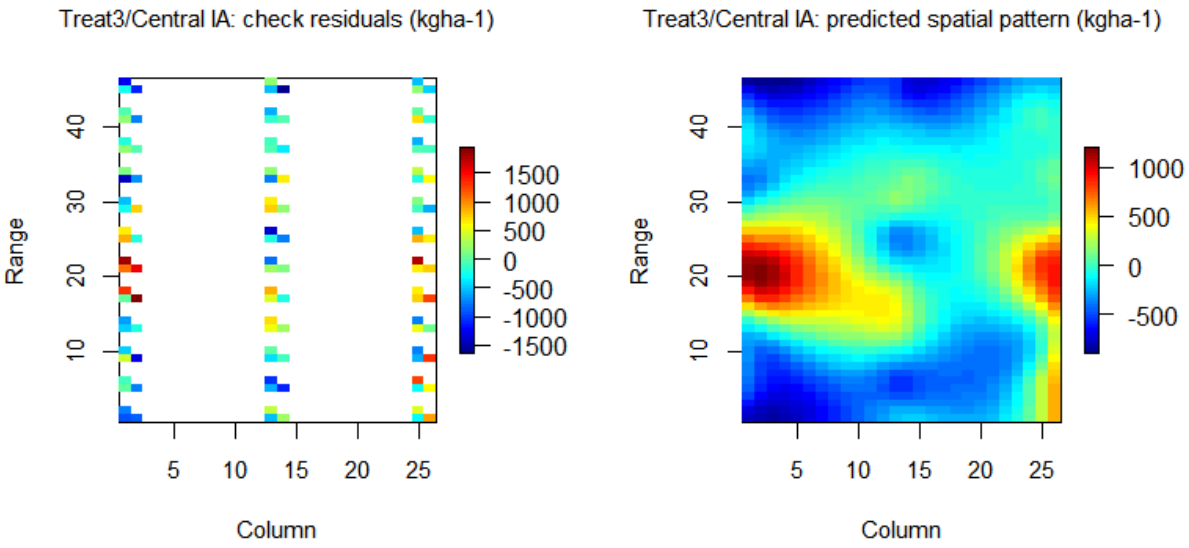

Fig. 3 Treatment 3 in Central IA. The heat map of check residuals estimated from the linear model with checks as fixed effects based on the yield of checks of the Spatial Study in 2013 (left), and the heat map of the predicted spatial pattern using the TPS spatial model based on the check residuals from the Spatial Study (right).

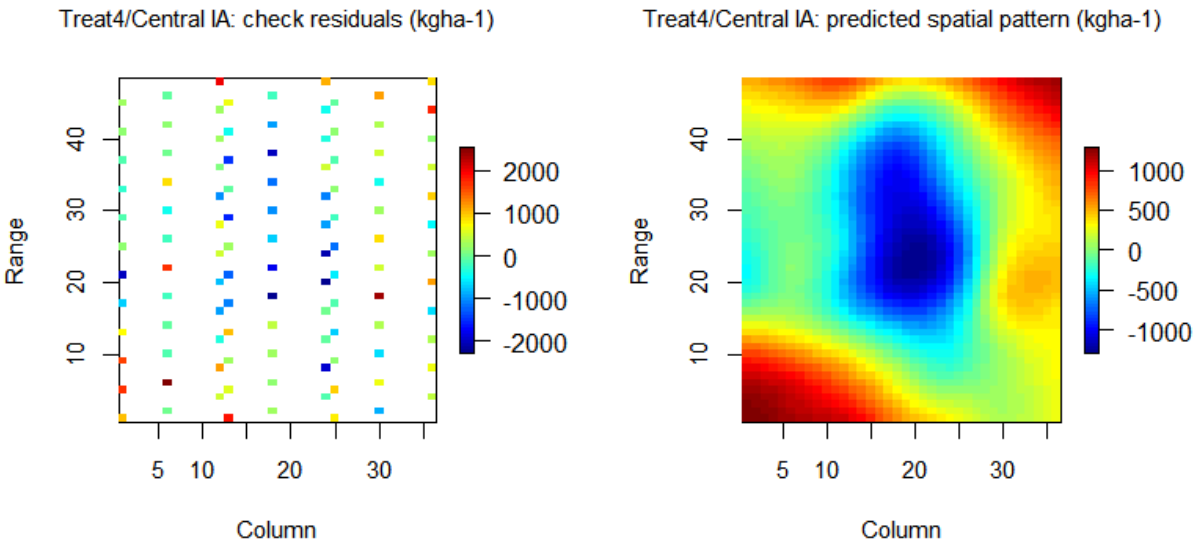

Fig. 4 Treatment 4 in Central IA. The heat map of check residuals estimated from the linear model with checks as fixed effects based on the yield of checks of the Spatial Study in 2013 (left), and the heat map of the predicted spatial pattern using the TPS spatial model based on the check residuals from the Spatial Study (right).

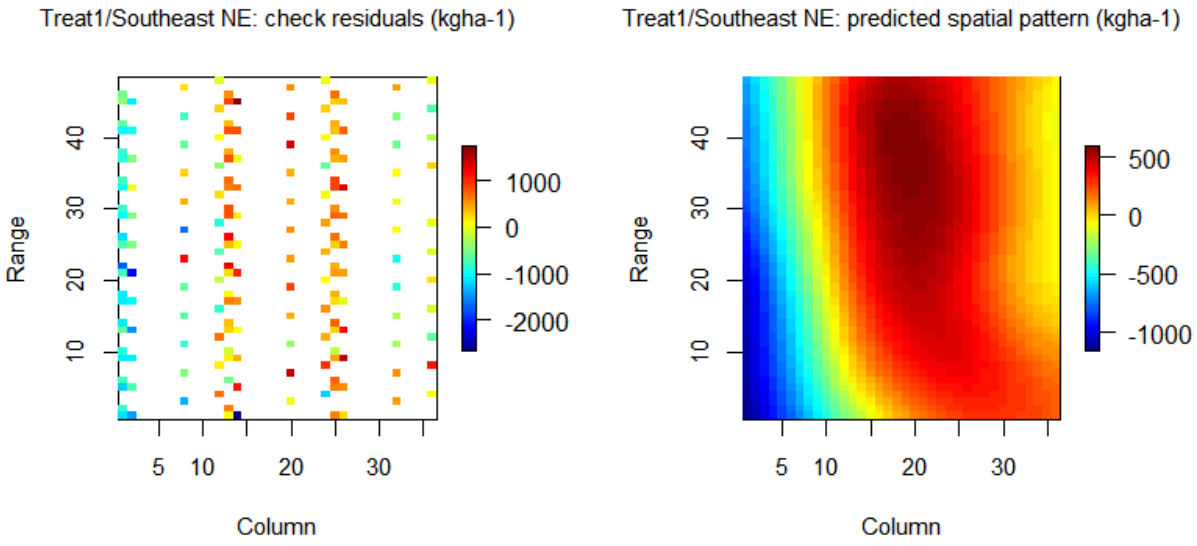

Fig. 5 Treatment 1 in Southeast NE. The heat map of check residuals estimated from the linear model with checks as fixed effects based on the yield of checks of the Spatial Study in 2013 (left), and the heat map of the predicted spatial pattern using the TPS spatial model based on the check residuals from the Spatial Study (right).

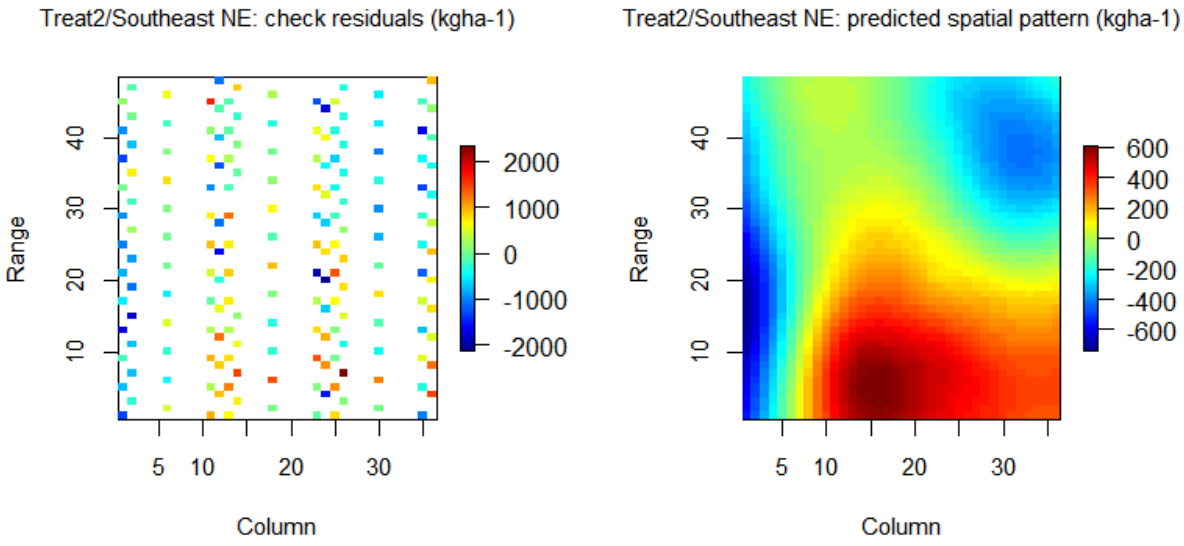

Fig. 6 Treatment 2 in Southeast NE The heat map of check residuals estimated from the linear model with checks as fixed effects based on the yield of checks of the Spatial Study in 2013 (left), and the heat map of the predicted spatial pattern using the TPS spatial model based on the check residuals from the Spatial Study (right).

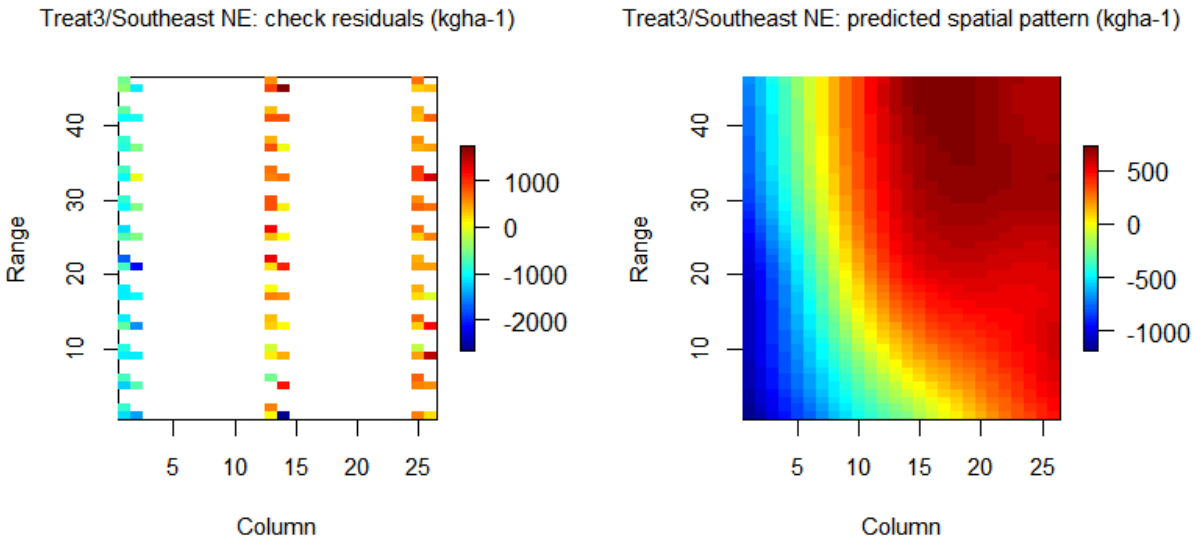

Fig. 7 Treatment 3 in Southeast NE. The heat map of check residuals estimated from the linear model with checks as fixed effects based on the yield of checks of the Spatial Study in 2013 (left), and the heat map of the predicted spatial pattern using the TPS spatial model based on the check residuals from the Spatial Study (right).

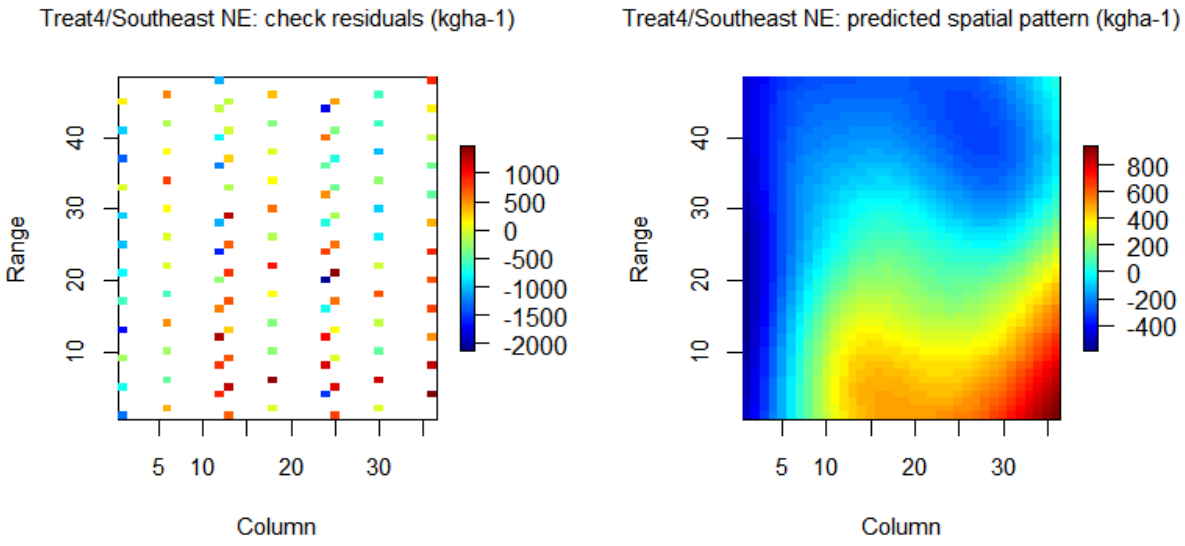

Fig. 8 Treatment 4 in Southeast NE. The heat map of check residuals estimated from the linear model with checks as fixed effects based on the yield of checks of the Spatial Study in 2013 (left), and the heat map of the predicted spatial pattern using the TPS spatial model based on the check residuals from the Spatial Study (right).

Fig. 9 Test-field 1 where treatments 1 and 3 were allocated in Central IA in the Spatial Study. The photo was taken on September 9, 2013. The plants with yellow leaves were underwent drought stress.

Fig. 10 Test-field 2 where treatments 2 and 4 were allocated in Central IA in the Spatial Study. The photo was taken on September 9, 2013. The plants with yellow leaves were underwent drought stress.

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CHAPTER 5. GENERAL CONCLUSIONS

The goals of this research were to improve the efficiencies of soybean field plot trials i) at early stages of progeny-row yield trials (PRYT) by experimental design and spatial modeling; ii) and at late stages of multiple environment trials by optimization of the multiple year and location trials (MYLT).

TPS Spatial Modeling Is an Effective Tool to Improve the Efficiency of Soybean PRYT Testing and Selection. A substantial spatial variation in soybean PRYT field could be present as evaluated by the Uniformity Study conducted with two commercial lines. In this experiment, the use of the TPS proved to be effective in reducing the error variance and the coefficient of variability, with an improvement in relative efficiency (IRE) of 37.9%. In the Early Generation Tests, 2565 lines were evaluated within test-sets along with three checks. The TPS model also was effective in the Early Generation Tests, the IRE was 40.4%. The correlation coefficients calculated between yield estimates obtained in the two-year Early Generation Tests and the Confirmation Study improved by 0.21 points compared to results from the non-TPS experiments. The results indicated that the use of the TPS spatial was effective in accounting for some of the spatial variation in field tests. However, limited by the number of checks used in the research, the adjustments obtained by the TPS were not effective in increasing the selection efficiency of the Early Generation Test on the basis of the individual line performance.

In the research conducted in 2013, the environmental conditions at the research site in Central IA from reproductive stage R3 to R6, and the growing conditions at the research site in Southeast NE damaged the filed experiments prevented the completion of this

research. Future research should be needed to explore the efficiency of the TPS spatial modeling on the basis of progeny-row plot in soybean PRYTs.

Two-year Multiple-location Trial Should Be Optimal to Capture the Soybean Top-yielding Lines with a Given Resource. The yield advantage of the lines selected from the PRYTs should be tested for the confirmation in multiple environment trials across years, and the superior lines with high-yielding and their best-fitting environments would be identified. The omnipresence of line x environment (GxE) interaction has been reported. However, little study regarding the decomposition of the variance of line x environment interaction was done. The knowledge of the compositions of GxE effect can and should be used to design optimal multiple year and location trials for accurate and stable prediction of line performance with the given resource. In this study, the results indicated that the predominant source of GxE variation is GxYxL. Along with the significant YxL, the significant GxYxL warranted multiple-year and multiple-location trials for sufficient predictability of line performance in the following year. However, two-year multiple-location trial should be sufficient to capture the soybean top-yielding lines. If a trial with relatively low presence of GxE, one-year multiple-location trials should be sufficient to capture the soybean top-yielding lines. In GXE25, the relatively lower presence of the GxE effect was observed, the future re-test of this group of lines in a different group of locations should be considered to confirm that the relatively lower presence of the GxE was not caused by sampling of the testing locations.

The improvement of the efficiency for field testing and selection is gaining the popularity across public and private research institutes.